# IMPACT OF ACUTE ANTIOXIDANT SUPPLEMENTATION ON NEURAL CARDIOVASCULAR CONTROL IN PSORIATIC SUBJECTS

A Thesis
by
NINA LAWRENE STUTE

Submitted to the School of Graduate Studies
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE DEGREE

May 2021
Department of Health and Exercise Science

# IMPACT OF ACUTE ANTIOXIDANT SUPPLEMENTATION ON NEURAL CARDIOVASCULAR CONTROL IN PSORIATIC SUBJECTS

A Thesis
by
NINA LAWRENE STUTE
May 2021

APPROVED BY:

Abigail Stickford
Chairperson, Thesis Committee

Stephen Ratchford
Member, Thesis Committee

Kevin Zwetsloot
Member, Thesis Committee

Kelly Cole
Chairperson, Department of Health & Exercise Science

Mike McKenzie, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

## Abstract

IMPACT OF ACUTE ANTIOXIDANT SUPPLEMENTATION ON NEURAL CARDIOVASCULAR CONTROL IN PSORIATIC SUBJECTS

Nina Lawrene Stute
B.S., The Ohio State University
M.S., Appalachian State University

Chairperson: Abigail S. L. Stickford, Ph.D.

**Abstract:** Recent studies indicate psoriasis not only affects the skin and joints, but, as a systemic inflammatory disorder, is also associated with increased risk of vascular complications leading to myocardial infarction and cerebrovascular stroke. Systemic inflammation is a known contributor to heightened cardiovascular disease risk. Individuals with psoriasis have been shown to have altered cardiovascular regulation; however, the underlying mechanisms are yet to be fully elucidated. Cellular oxidative stress can destabilize the cell and lead to cellular damage causing chronic inflammation. Ultimately, heightened oxidative stress and inflammation can lead to exaggerated muscle sympathetic nerve activity and vascular dysfunction-most notably decreased nitric oxide bioavailability-as well as chronic diseases such as hypertension, chronic obstructive pulmonary disease (COPD), and heart failure. Given the known inflammation associated with psoriasis, it is likely that oxidative stress and inflammation are major contributing factors to the increased risk for cardiovascular disease development in this population, potentially via alterations in vascular and autonomic function. However, due to the COVID-19 pandemic, a human subjects research study was unable to be conducted. Therefore, the purpose of this thesis was to perform a comprehensive literature review of inflammation and oxidative stress, as well as associated cardiovascular

risk, that is characteristic of psoriasis in order to a) design a research study to examine the potential amelioration of vascular and autonomic dysfunction via antioxidant supplementation in this population, b) submit a grant proposal to the National Psoriasis Foundation, and c) submit a Journal Club article to the *Journal of Physiology* expounding on the relationship between autoimmune diseases, autonomic (sympathetic) function, and cardiovascular risk.

## Acknowledgments

Thank you to my parents for affording me the opportunity of a graduate education. Thank you to my committee members; for your mentoring, patience, and flexibility during this pandemic. I learned so much from each of you! Lastly, thank you Dr. Abigail Stickford for your kindness and patience; I had a tough two years and you were exactly the mentor I needed.

## Table of Contents

## Layman's Statement

Psoriasis is an immune system disorder causing systemic inflammation. This inflammation impacts more than just the skin and joints; inflammation associated with psoriasis is detected in many different body systems. Psoriasis increases the risk of severe cardiovascular events, such as heart attack and stroke. The link between psoriasis and severe cardiovascular events has yet to be fully uncovered; this comprehensive literature review and subsequent study design will investigate the link between inflammation, cardiovascular events, and antioxidant supplementation in patients with psoriasis. Very few studies have investigated the nervous system's role in controlling the cardiovascular system in patients with psoriasis, let alone the possible impact of short-term antioxidant supplementation. This literature review and design of a research study will focus on how antioxidant supplementation might lower inflammation in blood vessels and improve cardiovascular function during rest and exercise in people with psoriasis. The aim of this review is to 1) investigate the role that psoriatic inflammation may play in nervous system control of the cardiovascular system, and 2) consider how antioxidant supplementation may impact blood vessel and nervous system function in this population. Further, the review will allow 1) the development of a research study design to quantitatively evaluate the role of inflammation in psoriatic disease, as well as 2) submission of a grant to fund the developed research study, and 3) submission of a Journal Club article to the *Journal of Physiology* on the complex interplay between autoimmunity, the nervous system, and cardiovascular disease. Ultimately, the insight gained from this review will help the scientific and health care communities understand the relationship between psoriasis and heart and blood vessel disorders.

# Chapter 1: Introduction

## Background

Psoriatic disease impairs the physical and psychosocial well-being of patients, leading to a decreased quality of life (Gudjonsson et al., 2004). The pathogenesis of this common disease that affects 2-3% of the U.S. population is not completely understood. Psoriasis presents with lesions that are characterized by T cell-mediated hyperproliferation of keratinocytes, causing unpleasant plaque formations on the skin (Gudjonsson et al., 2004). However, it has been well established that psoriatic disease, both psoriasis and psoriatic arthritis, affects more than just the skin and joints- it goes well beyond skin deep.

As a systemic inflammatory and autoimmune disease, psoriasis is associated with increased risk of severe vascular events such as myocardial infarction, cerebrovascular stroke, and atherosclerosis (Hu & Lan, 2017). Specifically, patients with psoriasis have a higher risk of myocardial infarction (Hazard Rate (HR) 1.21: 95% CI 1.10-1.32), atherosclerosis (HR1.28: 95% CI 1.10-1.48), and cerebrovascular stroke (HR 1.12; 95% CI 1.00-1.25) (Kaye et al., 2008) compared with age-matched individuals without psoriasis.

Along with the various health risks and decreased quality of life, psoriasis also poses a great economic burden on those diagnosed. The annual economic burden of psoriasis in the United States amounted to approximately $112 billion in 2013 (Brezinski et al., 2015). Many of the current therapies for treating psoriasis, such as cyclosporine, methotrexate, and other biological agents, can cost upwards of $368 per dose and are accompanied by a host of adverse side-effects. Current drugs used to treat psoriasis come with limitations: loss of efficacy, lack of affordability, and the health risks accompanied with immunosuppressants, such as higher susceptibility to infection, weight loss/gain, and muscular atrophy. These limitations warrant

the continued search for alternative solutions. Importantly, recent data show that antioxidants have been used to ameliorate oxidative stress and inflammation among other populations such as aging persons (Ratchford et al., 2019), hypertension (Sinha & Dabla, 2015), and heart failure (Georgiopoulos et al., 2017), giving credence to the notion that antioxidant supplementation may decrease systemic inflammation among patients with psoriasis as well. Antioxidants can be effective in treating and preventing disease given the correct diagnosis, subject, dose, and duration.

Systemic inflammation, like that seen in psoriasis, is a known contributor to heightened cardiovascular disease risk. Individuals with psoriasis have been shown to have altered cardiovascular regulation (Kaye et al., 2008); however, the underlying mechanisms are yet to be fully elucidated. While muscle sympathetic nerve activity has not been measured in this population yet, heart rate variability measures indicate heightened sympathetic outflow in this clinical population (Kaye et al., 2008). However, there are limitations to HRV; as you can't really isolate any one signal, or input (Hayano & Yuda, 2019).

There are, undoubtedly, a variety of factors that interact to initiate psoriasis, reach a chronic disease state, and unfold into various cardiovascular comorbidities. These factors, in no particular order, include: inflammation, oxidative stress, autonomic function/muscle sympathetic nerve activity (MSNA), adiposity, and various lifestyle factors such as diet and exercise habits. Investigating MSNA, inflammation, and oxidative stress is ff great theoretical importance to this investigation.

The role of oxidative stress in psoriatic patients (i.e.; chronic inflammation, like that seen in psoriasis), and cardiovascular comorbidities will be investigated in the present study. Inflammation seen in subjects diagnosed with psoriasis is not merely contained to the skin.

Psoriasis is a state of systemic inflammation, and these inflammatory pathways infiltrate aspects of homeostatic and allostatic control, mechanisms necessary to maintain life, including decreased exercise tolerance. Recent literature suggests increased oxidative stress plays a key role in the pathogenesis of psoriasis (Nestle et al., 2009). Reactive oxygen species (ROS) stem from by-products of cellular respiration and metabolic processes. When there is an imbalance favoring ROS, oxidative stress will be observed (Reczek & Chandel, 2015). Moderate amounts of ROS play an integral role as signaling molecules to regulate biological and physiological processes; however, high levels of ROS are known to cause histological pathology- cellular damage, DNA damage, and even cell apoptosis in extreme oxidative stress (Schieber & Chandel, 2014). Furthermore, oxidative stress-induced chronic inflammation can negatively impact exercise tolerance (Tsutsui et al., 2011) ultimately resulting in diminished quality of life and early increased risk of mortality (Myers et al., 2002). In fact, it has been shown that persons with high levels of chronic systemic inflammation and oxidative stress have a lower $VO_{2\ max}$, or the maximum amount of oxygen a person can utilize during exercise, than their healthy, age-matched counterparts (Rosado-Perez & Mendoza-Nunez, 2018). Fortunately, extreme ROS production is counterbalanced by an equally high rate of innate antioxidant activity in the body to maintain redox balance, at least in a non-compromised immune system.

Autonomic dysregulation is also a key factor in the development of cardiovascular disease and vascular events in many disease states. Compromised sympathetic outflow results in an increase in norepinephrine binding to α1 adrenergic receptors on the vascular smooth muscle, increasing $Ca^{2+}$ permeability, ultimately stimulating vascular smooth muscle vasoconstriction of the terminal arterioles. This vasoconstriction leads to increased peripheral

resistance, translating to increased blood pressure- which can result in a plethora of cardiovascular issues overtime. Accordingly, increased MSNA could be an underlying cause of cardiovascular events in psoriatic subjects.

Furthermore, vascular dysfunction is a hallmark aspect of the development of severe vascular events and disease states as well. Decreased nitric oxide bioavailability is the suspected root cause of this dysfunction, and, as such, flow mediated dilation will be used to investigate vascular function in the present study. Flow mediated dilation, which is dependent on shear stress induced endothelial release of nitric oxide, holds clinical significance in its ability to correlate with coronary artery function and cardiovascular disease risk (Inaba et al., 2010; Tremblay & Pyke, 2018). Wadley et al., proposed the vascular health triad, where inflammation and oxidative stress interact to predispose the vascular tree to damage, suspected by decreased nitric oxide bioavailability (Wadley et al., 2013). Endothelial function has been shown to be largely intact in psoriatic subjects when controlled for cardiovascular risk factors (Martyn-Simmons et al., 2011). In turn, when not controlling for cardiovascular risk factors (i.e.; hypertension, waist circumference, resting heart rate) subjects with psoriasis do display compromised vascular function (Jensen et al., 2011).

**Statement of the problem**

Psoriasis affects 2-3% of the United States population and presents with cardiovascular disease and severe cardiovascular event comorbidities. The underlying mechanisms behind psoriasis, the chronic inflammatory autoimmune disease itself, and the increasingly alarming incidence of cardiovascular issues associated with it, has yet to be fully elucidated.

As mentioned earlier, many of the current therapies for treating psoriasis are not only expensive but are accompanied by substantial adverse side-effects and limitations; such as higher susceptibility to infection, weight loss/gain, and muscular atrophy. Thus, alternative treatments may be economically and physiologically beneficial. While antioxidants alone are not a replacement for biologic drug therapy necessary to control psoriasis, adding antioxidants through supplementation could potentially attenuate cardiovascular disease risk in this population. Interestingly, recent data show that antioxidants can be effective in preventing and treating non-alcoholic steatohepatitis (a liver disease) (Sanyal et al., 2010) and in reducing cardiovascular events in individuals with type-2 diabetes, and given the correct diagnosis, subject, dose, and duration of treatment (Niki, 2014; Vardi et al., 2013).

**Purpose**

Due to the COVID-19 pandemic, a human subjects research study was unable to be conducted. Therefore, the purpose of this thesis was to perform a comprehensive literature review of inflammation and oxidative stress, as well as associated cardiovascular risk, that is characteristic of psoriasis in order to a) design a research study to examine the potential amelioration of vascular and autonomic dysfunction via antioxidant supplementation in this population, b) submit a grant proposal to the National Psoriasis Foundation, and c) submit a Journal Club article to the *Journal of Physiology* expounding on the relationship between autoimmune diseases, autonomic (sympathetic) function, and cardiovascular risk.

# Chapter 2: Literature Review

## Introduction

Psoriasis impacts many people in many different ways. As such, there are numerous ways to treat psoriasis; depending on the type and severity of the disease, and multiple lifestyle factors of the patient. This literature review will address aspects of the disease as it pertains to autonomic function, oxidative stress, and inflammation.

## Psoriasis

It has been well-established that psoriasis is an inflammatory autoimmune disorder; however, perhaps the most detrimental part of psoriasis is the plethora of comorbidities. These range from, but are not limited to, inflammatory bowel disease, metabolic syndrome, obesity, diabetes, and severe vascular events such as myocardial infarction and cerebrovascular stroke (Pearce et al., 2006). Myocardial infarction and cerebrovascular stroke are often observed in younger patients with more severe disease, ultimately contributing to the 3- to 4-year life expectancy reduction compared to healthy counterparts (Gelfand et al., 2006; Reich, 2012). The aforementioned comorbidities ultimately lead to decreased longevity and decreased quality of life in patients with psoriasis. Patients with psoriasis report their quality of life impairment equal to, or in some occasions, worse than cancer and severe heart disease patients (S. C. Weiss et al., 2002).

While psoriasis is more than skin deep, it is important to define the general anatomy where these pathologic skin lesions originate and take place. The skin consists of three tissue layer segments: the epidermis, dermis, and subcutis. The **epidermis** layer primarily consists of keratinocytes and the ever-important Langerhans cells (LC's), which are dendritic antigen

cells. LC's typically reside in the basal and suprabasal layers (Lowes et al., 2014). LC's specialize in antigen presentation and play a key role in the skin immune system. In an immune response (e.g. psoriasis) LC's can migrate to the local lymph nodes and present antigenic peptides to T cells, ultimately proliferating the antigen-specific immune response (Wang & Bai, 2020). The **dermis** layer is denoted by collagenous connective tissue to maintain integrity, blood vessels, and various types of immune cells. The dermis will also have sweat glands, hair follicles, and sebaceous glands. The erythema of psoriasis is due to angiogenesis via the signaling protein VEGF and dilated dermal blood vessels. The **subcutis** layer consists of a complex network of adipose tissue for protection and collagen cells. This layer of the skin houses larger blood vessels, which are integral in the immune response of psoriasis.

In healthy skin, keratinocytes undergo differentiation as they grow. In this differentiation process, the basal layer differentiates into spinous and granular keratinocytes. The granular layer keratinocytes express antimicrobial peptide molecules (AMP's), which are associated with innate immunity (Wang & Bai, 2020; Wang et al., 2018). This delicate balancing act gets disrupted in persons with psoriasis.

The diagnosis and treatment of psoriasis has evolved greatly over the past few decades. As the understanding of the disease has increased, so has the scope of prevalence and acknowledgement of comorbidities. Psoriatic manifestations on the dermis vary widely in severity and formation, which make it a difficult disease to fully evaluate and study, both from a histopathological and patient care point of view (Villasenor-Park et al., 2012). There are currently five different identified subtypes of psoriasis, all of which are diagnosed clinically - most without the need for skin biopsy and blood biomarkers. The severity of psoriasis is measured by the *Psoriasis Area and Severity Index* (PASI) (Schmitt & Wozel, 2005). The

PASI accounts for both severity and surface area and ranges from 0 (*no psoriasis*) to 72 (*extreme psoriasis*) (PASI: Appendix 1). Psoriasis is classified as "mild" with < 10% lesion surface area and as "moderate to severe" with >10% of lesion surface area along with a PASI score >12 points (Feldman, 2004).

The five identified subtypes of Psoriasis are as follows:

- Vulgaris or Plaque Psoriasis: the most common form of psoriasis, affecting 85-90% of those diagnosed (Griffiths & Barker, 2007). Plaque psoriasis is characterized by keratinocyte proliferation and the presence of vascular endothelial growth factor (VEGF) causing dermal cell vasculature angiogenesis. Plaque psoriasis is denoted mainly by acanthosis, or thickening of the epidermis seen in the red scaly plaque formations. It is typically observed on extensor surfaces like the knee and elbow (Griffiths & Barker, 2007). Dermatologists treat vulgaris/plaque psoriasis in numerous ways depending on comorbidities and various life-style factors.

- Guttate Psoriasis: mainly observed in a younger population (i.e., children and adolescents), Guttate psoriasis occurs when papules, a dry-elevated portion of skin, erupt on the anterior and posterior abdominal region post- tonsillitis and/or pharyngitis (beta-haemolytic streptococci), and in some cases following a viral infection. Guttate Psoriasis generally ceases on its own in approximately 3-4 months, though some dermatologists and physicians recommend antibiotics (Griffiths & Barker, 2007).

- Pustular Psoriasis: characterized by pustules instead of plaques. Pustular psoriasis has five known forms: generalized, localized, Von Zumbusch, palmoplantar, and acropustulosis. Systemic medications and phototherapy are commonly utilized to treat pustular psoriasis.
    1. Generalized Pustular Psoriasis is characterized by pustules with widespread erythema, or redness, accompanied by fever and/or malaise. Blood analysis is often utilized to diagnose Generalized Pustular Psoriasis; heightened C-reactive protein and certain elevated antibody levels are notably observed (Umezawa et al., 2003). Given the surface area for the area of affected skin, the threat of infection is of the utmost importance when treating generalized pustular psoriasis. As such, various biologics and immunosuppressants like cyclosporine, methotrexate, and infliximab/remicade (TNF- $\alpha$ blocker) (Robinson et al., 2012).
    2. Local Pustular psoriasis is characterized by the same pustules as generalized pustular psoriasis, but is localized to one area of the body. Again, underlying risk factors govern treatment, but various biologics and immunosuppressants like cyclosporine, methotrexate, and infliximab/remicade (TNF-$\alpha$ blocker) are oftentimes utilized (Robinson et al., 2012).
    3. Von Zumbusch pustular psoriasis can be life threatening and requiresimmediate medical attention. Pustules oftentimes appear almost instantaneously and then continue to dehydrate as the pustules dry-out. Patients with Von Zumbusch pustular psoriasis often need to be hospitalized to receive antibiotic and

hydration treatment; as the threat of infection is extremely high (Robinson et al., 2012).

4. Palmoplantar psoriasis is pustular psoriasis that presents on the soles of the hands and feet. This type of pustular psoriasis is generally treated with topical steroids and UV treatment (Robinson et al., 2012).
5. Acropustulosis presents are pustular psoriasis on the ends of the fingers. The eruption of acropustulosis is oftentimes preceded by injury to the area. Treatment includes topical steroids and systemic treatments, depending on severity and risk factors (Robinson et al., 2012).

- Inverse Psoriasis: rare form of psoriasis that appears in flexural skin folds (armpits, groin, etc). Inverse psoriasis exhibits the same inflammatory pathway as its well-known counterpart, plaque psoriasis, but differs in the primary locations (Syed & Khachemoune, 2011).

- Erythrodermic Psoriasis: a severe form of psoriasis that affects upwards of 75% of body surface area with inflammation. Erythrodermic psoriasis is the rarest subtype of psoriasis, impacting ~1% of those diagnosed. Patients diagnosed with Erythrodermic Psoriasis are at higher risk for congestive heart failure and various types of life-threatening infections compared to age-matched healthy counterparts. Many of those diagnosed with Erythrodermic psoriasis have severe relapses and require chronic aggressive systemic therapy, such as immunosuppressants and biologics (Rosenbach et al., 2010).

Overall, the basic pathological mechanisms of psoriasis are hypothesized to be mediated by communication between the dermal vascular cells, epidermal keratinocytes, T cells, and antigen presenting cells. As mentioned before, the exact pathogenesis of this crosstalk has yet to be fully elucidated, but it is suspected that increased proliferation of keratinocytes and certain endothelial cells, combined with rampant inflammation, conducts the hyperplasia of both the dermis and vasculature, resulting in the recognizable painful-plaques and lesions seen in various types of psoriasis (Ayala-Fontanez et al., 2016; Griffiths & Barker, 2007).

## Current Therapies for Psoriasis

While the knowledge and therapies for seemingly all chronic disease under the autoimmune umbrella is constantly evolving and updating thanks to good science, there are some current and well-known therapies for psoriasis that should be mentioned and explored in this literature review: biologics, immunosuppressants and cytotoxic drugs, as well at topical treatments.

*Biologics:*

Biologic agents are compounds manufactured from varying substances of living organisms- oftentimes these are lab cultured antibodies and proteins designed to aid or inhibit a specific signaling cascade in an immuno-reactions (Burkhart C & Goldsmith L., 2011). Biologic agents are oftentimes favored by some medical professionals for the treatment of psoriasis given their specificity in targeting cytokines that mediate inflammation (Burkhart C & Goldsmith L, 2011). Essentially, biologics are viewed as less systemically toxic than the alternative systemic therapies that are, by design, systemically immunosuppressive (Burkhart

C & Goldsmith L, 2011; Hu et al., 2018). To the authors knowledge, there many approved biologic agents to help treat psoriasis, in this literature review the five most common will be discussed.

*Alefacept (Amevive):* a T cell activation inhibitor, was the very first immunobiological agent approved to treat psoriasis of varying severities (Burkhart C & Goldsmith L, 2011; Frampton & Wagstaff, 2003). Alefacept is a lab manufactured human fusion protein. Certain portions of the Alefacept molecule binds with specific T cells, ultimately inhibiting the signaling cascade for T cell's activation involved with psoriatic eruption. Proven effective in clinical trials, the drug is administered by intramuscular injection weekly for approximately 12 weeks, followed by no injections for 12 weeks, and this process is continually repeated (Frampton & Wagstaff, 2003).

*Efalizumab (Raptiva):* also a T cell activation inhibitor, is another proven biologic agent for the treatment of psoriatic symptoms. This T cell inhibitor also disrupts the T cell activation by preventing the binding of certain molecules with vascular endothelial cells and other cells in the dermis and epidermis (Burkhart C & Goldsmith L, 2011; Weinberg, 2003). While this biologic therapy was thought to be effective and safe for treating psoriasis, the subcutaneous injections of the agent soon became associated with fatal brain infections via demyelination of neurons (Major, 2010). While Efalizumab is not classified as an immunosuppressant, it does dramatically modify immune function- which in this case, ended poorly and was withdrawn from the market in 2009 (Burkhart C & Goldsmith L, 2011; Major, 2010).

*Etanercept (Enbrel):* a tumor necrosis factor (TNF) inhibitor, works by blocking TNF-$\alpha$ which in turn decreases not only inflammation but also keratinocyte proliferation, and

vascular adhesion (Burkhart C & Goldsmith L, 2011). TNF-$\alpha$ is elevated in both the lesions and blood serum in patients with psoriasis. Specifically, Etanercept is a fully human TNF receptor fusion protein, inhibiting the action of TNF (Burkhart C & Goldsmith L, 2011; Krueger & Callis, 2004). Etanercept is administered via a pre-filled syringe which is to be inserted intramuscularly multiple times a week (Burkhart C & Goldsmith L, 2011).

*Infliximab (Remicade):* also a tumor necrosis factor (TNF) inhibitor, is a manufactured mouse-human antibody that binds to TNF-$\alpha$ (Burkhart C & Goldsmith L, 2011). Infliximab is one of the more popular biologics used to treat psoriasis, however since is not fully human the risk of developing neutralizing antibodies is slightly greater than its fully-human counterparts. Infliximab is administered via intravenous infusion every 6-8 weeks (Burkhart C & Goldsmith L, 2011). Infliximab is widely used for other autoimmune diseases as well, including but not limited to Crohns disease, ulcerative colitis, eosinophilic esophagitis, and rheumatoid arthritis.

*Adalimumab (Humira):* also a tumor necrosis factor (TNF) inhibitor, is a fully-human antibody that binds TNF-$\alpha$. As aforementioned, adalimumab has slightly less risk associated with it than Infliximab, as it is fully-human. Adalimumab is administered via intravenous infusion biweekly (Burkhart C & Goldsmith L, 2011). Adalimumab is also very popular for the treatment of adult Crohns disease.

*Immunosuppressive and Cytotoxic Treatments*

*Methotrexate:* a chemotherapy and immunosuppressive drug. Methotrexate works by suppressing immunocompetent cells in the skin, but its systemic qualities as an immunosuppressant should not be overlooked. Methotrexate comes bearing dangerous side effects and interactions; it has the ability to cause “anti-proliferative effects” on bone marrow, as it is also used to treat leukemia (Burkhart C & Goldsmith L, 2011). The risk of Methotrexate-

inducted hepatic fibrosis (the first stage of liver scarring) is extremely serious and is more prevalent in patients being treated for psoriasis than other diseases (Burkhart C & Goldsmith L, 2011; Heydendael et al., 2003). Complete blood counts need to be completed regularly throughout methotrexate treatment.

*Azathioprine (Imuran):* in dermatological practice is used for inflammatory skin conditions, one being psoriasis (Burkhart C & Goldsmith L, 2011). This immunosuppressant is most commonly used among kidney transplant patients to prevent rejection, as well as for Crohns disease and ulcerative colitis, much like methotrexate. Azathioprine is commonly used in combination with Infliximab for various autoimmune disorders (Burkhart C & Goldsmith L, 2011; Colombel et al., 2010).

*Cyclosporine (Neoral, Gengraf):* an immunosuppressant that is isolated from the fungus called tolypocladium-inflatum. Known for its ability to inhibit calcineurin, which is responsible for the transcription of various cytokines- which ultimately results in inhibition of T cell activation (Burkhart C & Goldsmith L, 2011). Calcineurin is also present in Langerhans cells (LC), LC's specialize in antigen presentation and play a key role in the skin immune system. In an immune response (e.g. psoriasis) LC's can migrate to the local lymph nodes and present antigenic peptides to T cells; ultimately proliferating the antigen-specific immune response (Wang & Bai, 2020). Calcineurin's presence in LC's could partially explain its efficacy in treating psoriasis.

As with most systemic immunosuppressants, there are wide spread risks and adverse effects. Cyclosporine, while effective in treating mild to severe forms of psoriasis is known to cause renal dysfunction and hypertension. Creatinine levels should be continuously monitored, as well as blood pressure (Burkhart C & Goldsmith L, 2011; Flores & Kerdel, 2000).

*Tazarotene (Tazorac):* is a topical retinoid used for the treatment of psoriasis. A retinoid is defined as a compound, either synthetically manufactured or natural, that displays vitamin-A like activity, which impacts regulation of cell proliferation (Burkhart C & Goldsmith L, 2011). Tazarotene is helpful in the treatment of psoriasis because it results in the inhibition of keratinocyte proliferation, as well as altered gene expression of inflammatory cytokines associated with psoriasis (Heath et al., 2018).

While this list of biologic agents, immunosuppressants, cytotoxic drugs, and topical retinoids is far from all encompassing, it highlights the risks to the patients and physician-labor associated with the treatments.

**Inflammation and Proposed Mechanisms of Psoriasis**

> *"When the augmentation of the natural excitability is attended by pain, redness, and swelling, it is termed inflammation. This metaphorical expression, invented in the infancy of science, was originally intended to represent a morbid state in which the part affected appeared as if they had been submitted to the action of fire. As it was adopted into medical language without having any precise or well-defined idea attached to its signification, […] it has now become so vague and in its interpretation so arbitrary, that it has really lost all value as a term of science; and like an old coin, should be forthwith withdrawn from circulation, calculated to produce constant error and confusion."*
>
> – *G. Andral, 1832* ("A Treatise on Pathological Anatomy," 1831)

The inflammatory pathways involved in psoriasis are complex and variable. Genetic predispositions and environmental factors like stress, trauma to the skin, certain drugs, smoking, and other risk factors can all trigger the inflammatory cascade. Importantly, inflammation is known to be a common denominator in many conditions and diseases.

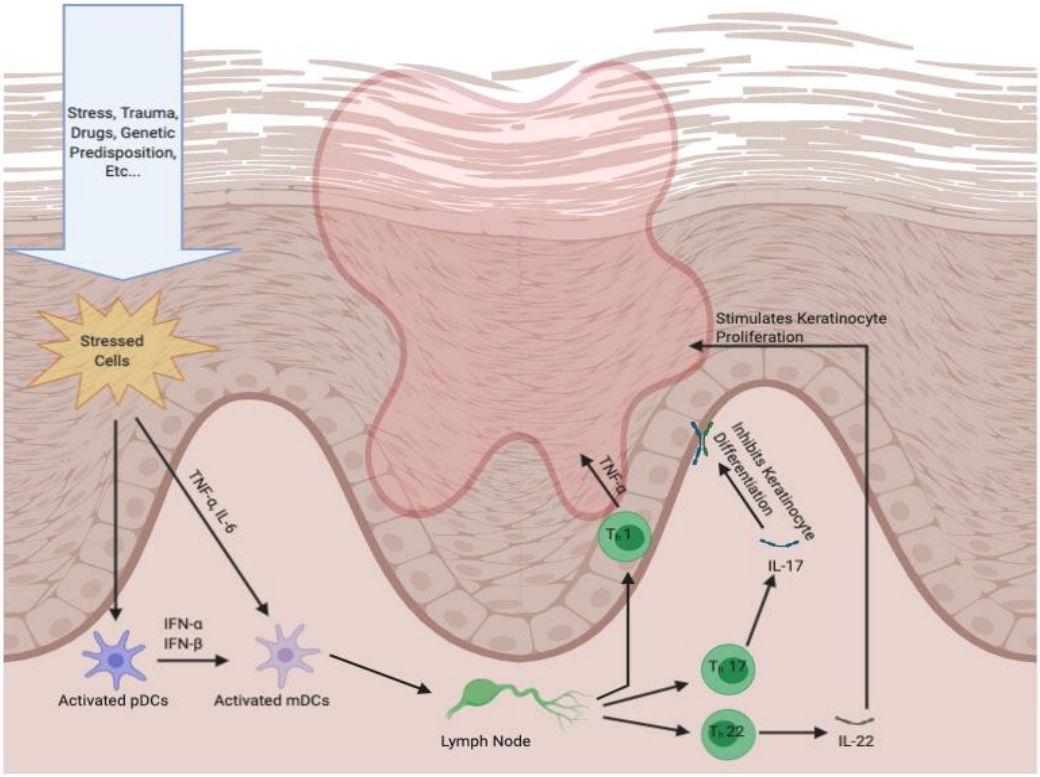
Type 1 Interferons (INFs), INF-a and INF-b, and activated plasmacytoid dendritic cells (pDCs) are present in psoriatic skin. INF-a and INF-b's production of activated pDCs enables the myeloid dendritic cells (mDCs) to spark the cascade of activated T cell subsets. mDCs and pDCs (plasmacytoid Dendritic cells) from the stressed/damaged epidermis not only play a role in secreting the necessary IFN's, but also release certain Interleukins; IL-23 and IL-12 to activate IL-17- resulting in the production of T cells and T helper ($T_h$) cells. T cells, formed in the thymus gland, are an essential part of the immune response. $T_h$ cells are a type of T cell that is essential in the adaptive immune system, responding to perturbations in the homeostatic state (Eberle et al., 2016).

T cells and $T_h$ cells ($T_h$ -1, $T_h$-17, and $T_h$-22) then stimulate the production of the well-known inflammatory cytokines IL-1, IL-6, IL-17, and TNF-a, which mediate effects on

keratinocytes, subsequently driving psoriasis (Eberle et al., 2016; Lowes et al., 2014; Reich, 2012). These immune processes can be seen in Figure 1. Angiogenesis will occur in moderate to serve cases of psoriasis, making its chronic state of inflammation easier to maintain and expand. Accordingly, psoriatic severity is positively correlated with VEGF levels (Bhushan et al., 1999).

**Figure 1**

*Psoriatic lesion.*




*Note.* T Helper (Th) cells stimulating production of inflammatory cytokines, driving psoriatic lesions.

Inflammation from these cytokines is not merely contained to the skin. Rather, psoriasis is a state of systemic inflammation, and these inflammatory pathways infiltrate aspects of

homeostatic and allostatic control, mechanisms necessary to maintain life, all the way down to decreased exercise tolerance. Research indicates that persons with high levels of chronic systemic inflammation and oxidative stress have a lower $VO_{2\ max}$ than their healthy-age matched counterparts (Rosado-Perez & Mendoza-Nunez, 2018). These findings suggest that systemic inflammation and oxidative stress may reduce exercise tolerance/capacity in those with psoriasis.

**Oxidative Stress: A Proposed Mechanism of Psoriasis**

Oxidative stress is an imbalance between oxidants, in this case reactive oxygen species (ROS), and antioxidants, favoring the oxidants. This pro-oxidant shift is evident in many disease states, but also prominent in advancing age (Wadley et al., 2013) (ROS> antioxidants). ROS, highly reactive free radical species, are molecules with unpaired electrons in their respective valence shells. The pro-oxidant shift leads to a disruption in redox signaling/balance, in some cases causing molecular damage. In healthy tissue, antioxidants are able to largely combat the ill effects of ROS; however, increased oxidative stress can nullify ROS defense mechanisms (Zhou et al., 2009).

This weakened defense mechanism could be partly to blame for the proliferation of psoriasis-related inflammation and lesions. In other words, a cellular redox imbalance could play a significant role disrupting skin homeostasis and in the development of a pathological skin environment, such as psoriasis (Briganti & Picardo, 2003; Zhou et al., 2009). While ROS are derivatives of normal and life-sustaining biological process (e.g., cellular oxidation processes), redox signaling balance is important. ROS have the ability to act as second

messengers to modulate various transcription factors across a wide array of inflammatory pathways.

Oxidative stress is subsequently involved in many DNA transcription and translation modifications, as well as production of inflammatory cytokines. These oxidative mechanisms underlie the pathophysiology of psoriasis, as well various cardiovascular comorbidities like atherosclerosis and cerebral stroke (Armstrong et al., 2011; Wadley et al., 2013).

With these transcriptional and translation changes, there is a subsequent increase in angiogenesis, caused by vascular endothelial growth factor (VEGF) in the dermis and epidermis; as mentioned previously, this is a hallmark sign of psoriatic skin. The upregulation of VEGF causes increased vascularity and blood supply that will support the thick and dense keratinocyte layer, increasing circulating lymphocytes into the lesioned areas. Recent studies show that ROS can induce angiogenic pathways (Lin & Huang, 2016; West et al., 2010).

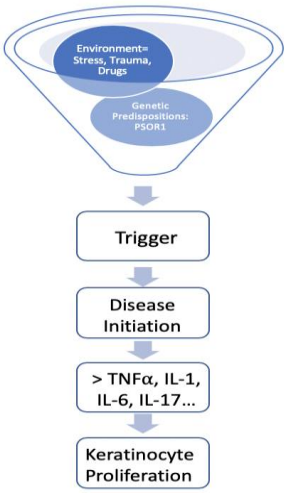
One of those pathways of interest in psoriasis would be the MAPK pathway. MAPKs play a vital role in cell differentiation and proliferation in immune responses (Lin & Huang, 2016). Increased p38MAPK, along with other MAPKs, has been shown to be increased in psoriatic epidermal lesions (Yu et al., 2007). Hence, ROS may play a large role in the drive of psoriasis pathogenesis (Popov & Lewin, 1991).

As the literature surrounding oxidative stress in various inflammatory disorders has grown, so has the use of antioxidant supplementation in an effort to curb ill effects of oxidative stress (Wadley et al., 2013). While the antioxidants counterbalance alone are not suggested for treatment of chronic disease, recent work is suggesting that antioxidant supplementation when implemented correctly at a suitable dosage with no medication interactions, may prevent and

help curb symptoms of psoriasis (Lin & Huang, 2016; Niki, 2014). Additionally, increased oxidative stress is an independent risk factor for cardiovascular disease (Wadley et al., 2013).

In a landmark paper by Wadley et. al, the interactions between oxidative stress and

**Figure 2**
*Triggers of psoriasis*




*Note.* Environmental and genetic factors triggering disease initiation and influx of inflammatory cytokines.

inflammation were examined to culminate a proposed hypothesis of the "vascular health triad" (Wadley et al., 2013). This review brings strong evidence that both oxidative stress and inflammation interact and predisposes the vasculature to damage. This vascular dysfunction is suspected to transpire through numerous mechanisms, but the most notable being reduced nitric oxide (NO) bioavailability, a vasodilator (Wadley et al., 2013). Furthermore, oxidative stress and inflammation have been linked independently to cardiovascular comorbidities (Dhalla et al., 2000; Ross, 1999). The inflammatory cytokines in question with regards to vascular dysfunction, are the same that are notably overproduced in patients with psoriasis: TNF-$\alpha$, IL-1, IL-6, CRP. Oxidative stress and inflammation are known to increase with age, regardless of disease state. Therefore, an aging population in a disease state that involves high

levels of inflammation, like that seen in psoriasis, deserves special consideration and investigation.

**Cardiovascular Comorbidities**

The literature supports the hypothesis that psoriasis results from a complex interaction between an individual's genetic susceptibility and various environmental factors (Figure 2). Work by Griffiths has identified upwards of 20 psoriasis-susceptibility gene loci, as well as genes that play a fundamental role in the pathogenesis of the disease. The *PSORS1* the susceptibility gene near the antigen *HLA CW6* appears to be particularly important (Griffiths & Barker, 2007). Genetic predisposition combined with an environmental trigger, such as tissue injury, known as the Köebner phenomenon, or infection of beta-haemolytic streptococci (guttate psoriasis) are highly associated with development of psoriasis (G. Weiss et al., 2002).

Perhaps the most detrimental aspect of psoriasis is the plethora of comorbidities. These range from, and are not limited to, cardiovascular disease (CVD), severe cardiovascular events (e.g., myocardial infarction and stroke), obesity, diabetes, metabolic syndrome, and inflammatory bowel disease (IBD) (Pearce et al., 2006). The many comorbidities make isolating the impact of psoriasis on health outcomes difficult, however studies that have controlled for some of these factors indicate that individuals with severe psoriasis are still at an increased risk of early mortality, especially younger patients (Abuabara et al., 2010; Ahlehoff et al., 2012; Mehta et al., 2011).

Work by Gelfand and Ludwig show that younger psoriatic patients are twice as likely to have plaques in the coronary arties than age-matched healthy adults, and accordingly, are three times more likely to suffer from myocardial infarction (Gelfand et al., 2006, 2007; Ludwig

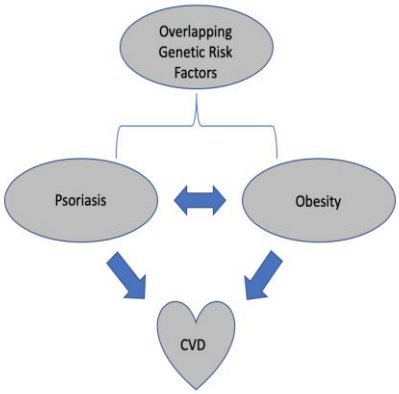
et al., 2007). These findings suggest that psoriasis may be an independent risk for myocardial infarction and coronary heart disease. Again, the mechanism for this risk may be related to oxidative stress and inflammation-mediated declines in NO bioavailability, ultimately limiting the vasodilatory capacity.

## Psoriasis and Obesity: The Unavoidable Confounding Variable

Those diagnosed with psoriasis are typically of higher adiposity than non-psoriatic individuals, the interaction between the obese state and psoriasis should be discussed. Patients with psoriasis are at a >50% increased odds of being obese than the general population, with the majority being overweight (Armstrong et al., 2012).

Common inflammatory pathways seen in CVD/vascular events, insulin resistance, adipokines, oxidative stress, and angiogenesis may explain the correlation

**Figure 3**
Overlapping factors of psoriasis




*Note.* Simplified schematic of complex interplay of overlapping genetic risk factors between psoriasis, obesity, and development of cardiovascular disease.

between psoriasis and various cardiovascular and metabolic disorders (Hu & Lan, 2017). However, the most commonly shared characteristic between psoriatic and cardiometabolic patients is increased adiposity.

Obesity itself is an independent risk factor for cardiovascular disease and numerous of adverse health outcomes (Hubert et al., 1983). The majority of patients diagnosed with psoriasis are above normal weight (Gisondi et al., 2007; Puig, 2011), and individuals with psoriasis have higher body mass index (BMI) than healthy control subjects (Duarte et al., 2013; Miller et al., 2013; Mysliwiec et al., 2017). Work by Duarte et. al. has also shown a dose-dependent relationship between the severity of psoriasis and waist-to-hip ratio and waist circumference, mainly indicating central obesity, in particular, is associated with psoriasis (Duarte & Silva, 2014). This observed relationship adiposity and psoriasis severity could be due to a complex interaction of factors, including decreased exercise tolerance from both psoriasis and/or the myriad of comorbidities, unhealthy lifestyle habits and/or socioeconomic status.

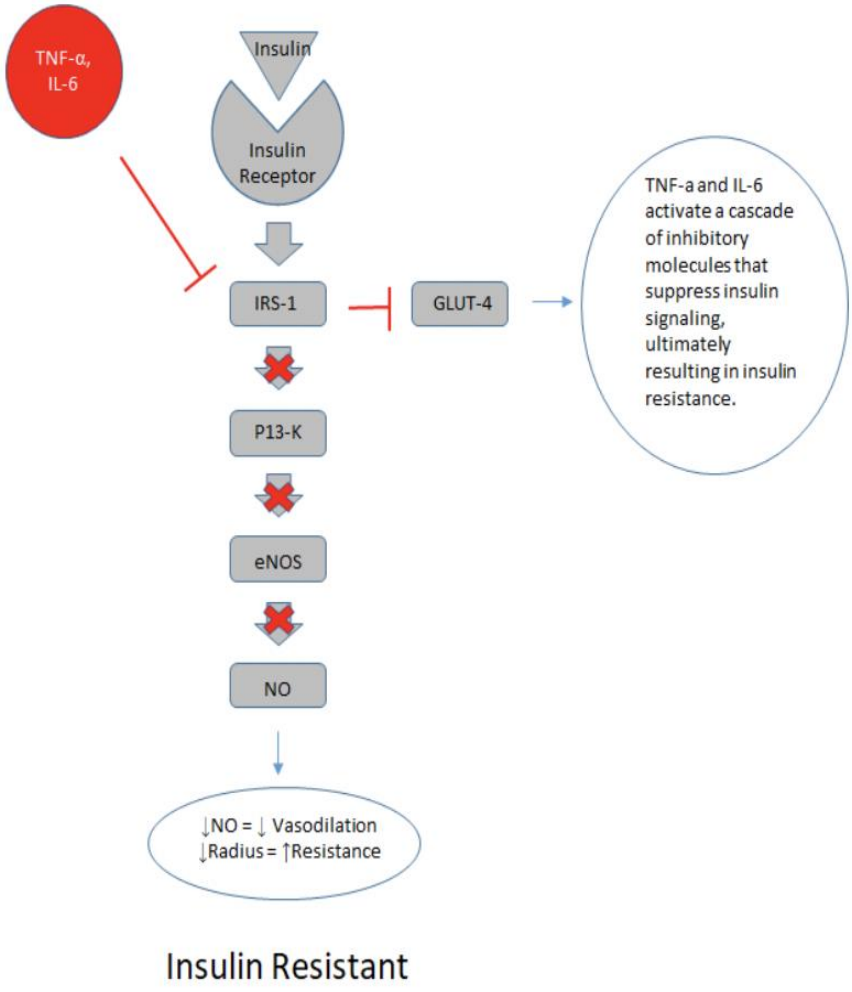
Beyond the overall associations seen between obesity and psoriasis, there are overlapping genetic predispositions as well (Figure 3). Recent studies demonstrate that the same co-morbidities are present in those diagnosed with psoriasis across all ages, ethnicities, races, sex, and genders - suggesting that these overlapping genetic predispositions result in shared comorbidities/risk factors (Augustin et al., 2015; Augustin et al., 2010; Reich, 2012; Tsai et al., 2011). The relationship between psoriasis and obesity/metabolic syndrome has been a topic of conversation in the current literature. It is well-established that adipocytes, specifically in central adiposity, are essential in the production of pro-inflammatory cytokines

(TNF-a, CRP) from adipocytes, specifically in central adiposity (Duarte & Silva, 2014; Meijer et al., 2011). Central adipocytes essentially act as an endocrine organ in this regard.

A paper of great theoretical importance by Boehncke et. al. introduced the possibility of the "Psoriatic March." Boehncke states that with the compiling evidence of the systemic nature of psoriasis, there is evidence to support a causal link between psoriasis and cardiovascular disease/severe cardiovascular events. Boehncke argues that the systemic inflammation of this autoimmune disease, which is primarily seen in obese persons, may directly trigger insulin resistance: causing endothelial cell dysfunction and subsequently leading to atherosclerosis. Similar to the work done by Wadley et. al, these proposed stepwise factors, with decreased NO bioavailability being a central theme, ultimately culminate to an increased risk of developing cardiovascular disease and/or leading to a severe vascular event (Boehncke et al., 2011; Wadley et al., 2013).

**Figure 4**

*Insulin resistance and vascular function.*




*Note.* Insulin resistance impacting NO bioavailability.

With obesity being an unavoidable confounding variable in the majority of subjects with psoriasis, insulin sensitivity cannot be ignored in the literature review. While measuring insulin sensitivity is outside the scope and budget of this project; insulin resistance should not be overlooked (Figure 4). Insulin resistance**,** or reduced glucose uptake, also plays a role in endothelial dysfunction by way of decreased blood flow and decreased vasodilation (i.e., ↓NO) (Lago et al., 2008). NO is crucial in regulating vascular tone and fighting vascular disease. NO is synthesized by way of endothelial nitric oxide synthase (eNOS). eNOS is the predominant

isoform in the vasculature, and thus is responsible for the majority of NO production in endothelial tissue (Forstermann & Munzel, 2006).

NO also has the ability to decrease inflammation by inhibiting white blood cell accumulation on the blood vessel wall, which could potentially help protect the vasculature from atherosclerosis (Boehncke et al., 2011).

It is clear that the antiatherogenic effects of NO are invaluable, NO dependent vasodilation is a necessary factor for vascular health. Both Boehncke's and Wadley's reviews provide compelling evidence for the damage that chronic inflammation can ensue on the vasculature in psoriasis. However, with so many confounding comorbidities in chronic inflammatory diseases like psoriasis, it will be near impossible to isolate such independent relationships. The relationship between psoriasis and CVD remains to be fully elucidated.

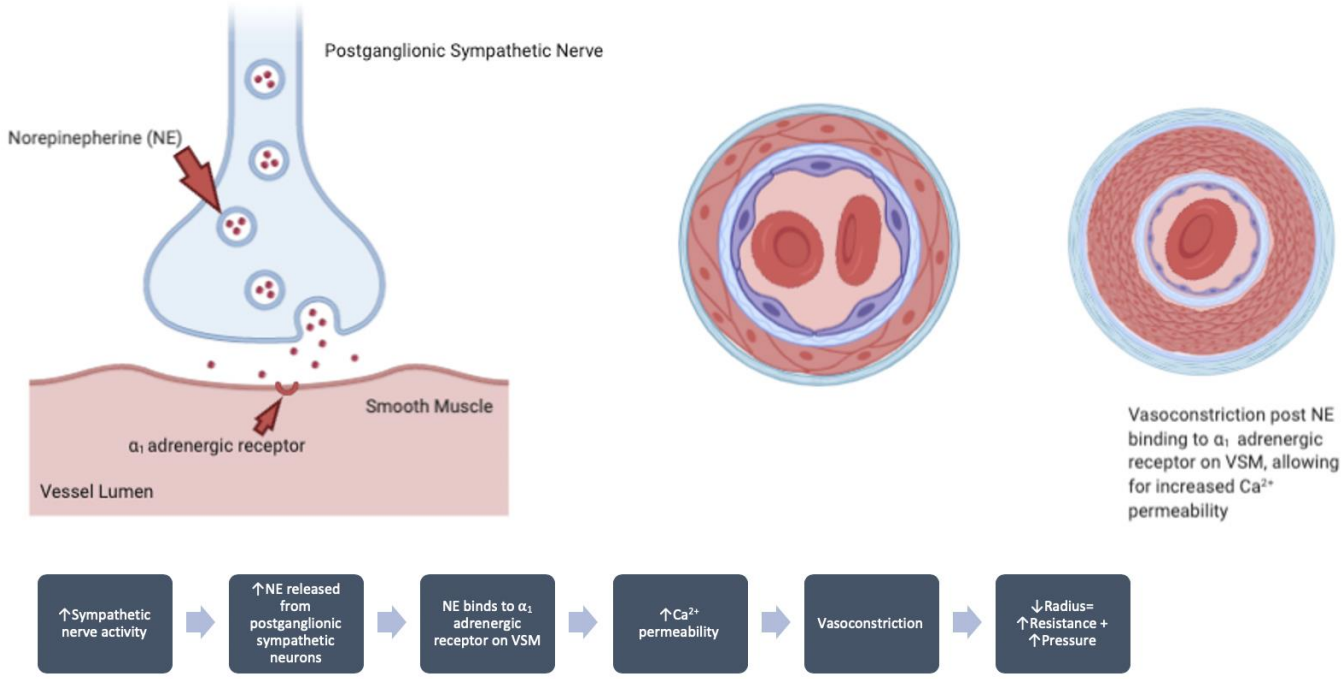
**Sympathetic Activity and Inflammation**

Oxidative stress and inflammation also influence the autonomic nervous system. The autonomic system regulates various life sustaining activities, such as digestion, blood pressure, heart rate, respiration, and so on. The autonomic nervous has three distinct divisions; sympathetic, parasympathetic, and enteric. The sympathetic nervous system, in particular, is of great importance in the context of psoriasis and adverse cardiovascular outcomes.

The sympathetic nervous system plays a large role in controlling the cardiovascular system. In a non-diseased person, the "fight or flight" response is activated in times of stress. However, in a variety of disease states this "fight or flight" response of norepinephrine (NE) release can become sustained to a certain degree. NE is released from sympathetic neurons and binds to $\beta - 1$ adrenergic receptors on the sinoatrial and atrioventricular nodes of heart, causing more frequent heart contractions.

NE does more than just bind to receptors on the myocardium. NE will bind to $\alpha 1$ adrenergic receptors on the vascular smooth muscle, which stimulates vasoconstriction; increasing blood pressure (Figure 5). The sympathetic nervous system is integral to optimal regulation of the cardiovascular system (Macefield, 2013), and – accordingly - altered sympathetic neural activity is associated with disease risk (Barretto et al., 2009; Malpas, 2010; Vallbo et al., 2004). Increases in resting and reactive sympathetic activity can have detrimental effects on several physiologic systems, including alterations in cardiac contraction (Moreira et al., 2017) and impairments in vascular function (Thijssen et al., 2006). Furthermore, sympathetic overdrive has been identified as an independent predictor of mortality in a number of diseases (Brunner-La Rocca et al., 2001; Grassi et al., 2015; Grassi et al., 1995; Holwerda et al., 2019; Huggett et al., 2003; Taylor et al., 2014).

**Figure 5**
*Muscle sympathetic nerve activity.*




*Note.* NE binding to alpha 1 adrenergic receptor on VSM, stimulating vasoconstriction.

Sympathetic activity is increased with excess adiposity, a state of inflammation indeed, in otherwise healthy individuals in urinary and plasma NE spillover, efferent postganglionic muscle sympathetic nerve activity (MSNA), and renal NE spillover compared to normal weight individuals (Huggett et al., 2004; Lambert et al., 2010; Lambert et al., 2007; Rahmouni et al., 2005). More specifically, increased body fat percentage has been directly correlated to sympathetic overactivity, with resting MSNA levels in obese persons 50% higher than in healthy matched counterparts, some researchers found (Alvarez et al., 2002; Sivenius et al., 2003). The underlying mechanisms behind the increased sympathetic activity observed in obese persons is due to a complex interaction of many factors (Alvarez et al., 2002; Grassi et al., 2004; Sivenius et al., 2003).

Increased MSNA is a key factor in the development of cardiovascular diseases and events. Increased MSNA results from in an increase in norepinephrine, among other neurotransmitters, binding to α1 adrenergic receptors on the vascular smooth muscle, increasing $Ca^{2+}$ permeability, and ultimately stimulating vascular smooth muscle vasoconstriction, or contraction, of the terminal arterioles (Figure 5). This vasoconstriction leads to increased resistance, translating to increased blood pressure. Generally, this mechanism is essential for controlling blood pressure. However, elevated sympathetic outflow, especially later in life, can ultimately result in a plethora of cardiovascular issues. Increased MSNA is a plausible contributing factor in the development of cardiovascular disease and vascular events in psoriatic subjects. The measurement of MSNA may also prove to be clinically relevant, as is it a strong predictor of CVD in older adults regardless of disease state.

The mechanisms behind why obesity correlates with increased sympathetic activity has yet to be fully uncovered. A landmark paper done by Landsberg in 1986 suggests the ongoing response to a positive energy balance results in an allostatic response wherein there is an increase in **β**-adrenergic thermogenesis to prevent further proliferation of adipose tissue. This accumulation of adiposity results in sympathetic nervous system activation in part of that allostatic response (Landsberg, 1986).

There is also emerging evidence that adipokines expressed in central adiposity itself can contribute to sympathetic overdrive (Abuabara et al., 2010; Smith & Minson, 2012). TNF-a, IL-6, and CRP can act as messengers to upregulate other harmful adipokines (Smith & Minson, 2012), in turn increasing sympathetic activity.

In obesity driven hyperinsulinemia, the sympathetic nervous system may also be activated by the hypothalamic-pituitary-adrenal (HPA) axis (Anderson et al., 1991; Berne et

al., 1992; Vollenweider et al., 1995). Hyperinsulinemia is shown to enhance the sensitivity, or gain, of the arterial baroreflex control of MSNA (Anderson et al., 1991).

The arterial baroreflex is the first line of defense when it comes to regulating acute blood pressure changes. The baroreceptors located in the carotid sinus and aortic arch are mechanoreceptors that respond to stretch and pressure in the arterial walls. Once a mechanical deformation is detected, the baroreflex will initiate the appropriate response either vasculature control (sympathetic) and/or cardiac control (cardiovagal) (Fadel et al., 2003; Fu & Ogoh, 2019). For example, a sudden increase in blood pressure, like that brought on during a Valsalva maneuver, will trigger the mechanoreceptors, which will signal to the medulla oblongata where sympathetic drive will be inhibited, allowing acetylcholine to be released to slow the HR and relax the peripheral vasculature (La Rovere et al., 2008). The sympathetic aspect of the baroreflex, MSNA, can be recorded directly via microneurography.

It is easy to recognize how decrements in the baroreflex could compound to serious health implications. Cardiovascular diseases are more often than not chaperoned by baroreflex sensitivity issues where a chronic adrenergic activation is observed (La Rovere et al., 2008). When the baroreflex is impaired, a resultant chronic increase in MSNA can be observed, increasing risk of cardiovascular disease and vascular events. When the comorbidities seen in psoriasis, like obesity (where sympathetic baroreflex is impaired/attenuated), are added to the mix, the risk is elevated even further (Grassi et al., 1995).

**Sex and Age Differences**

Interestingly, autoimmune diseases have greater incidence in females than males. This is suspected to be due to the larger number of genes in the X chromosome; ultimately increasing the probability of a mutation occurring given that females have two X chromosomes (Angum et al., 2020). While psoriasis does impact a greater number of females than males, interestingly females tend to have lower PASI severity scores especially in regards to cardiovascular disease risk (Garshick et al., 2019; Hägg et al., 2017). The reason(s) for these sex differences in incidence and severity are likely genetic and/or hormonal in nature (Angum et al., 2020). Additionally, there are well established age-related changes in sympathetic activity; specifically, MSNA increases significantly with age in both males and females (T. Matsukawa et al., 1998), which may further impact the severity of and cardiovascular risk from psoriasis. Notably, the changes in sympathetic outflow and subsequent changes in vascular resistance and blood pressure with aging differ between the sexes. Females exhibit a greater increase in MSNA with age due to menopause (Casey et al., 2007; Tanaka et al., 1998), as changes in estrogen and progesterone can affect sympathetic neural outflow and neurovascular transduction (Hart et al., 2013; Weitz et al., 2001). These known age and sex differences independently elevate cardiovascular disease risk in otherwise healthy older individuals, but may be particularly relevant in older adults with pro-inflammatory diseases like psoriasis.

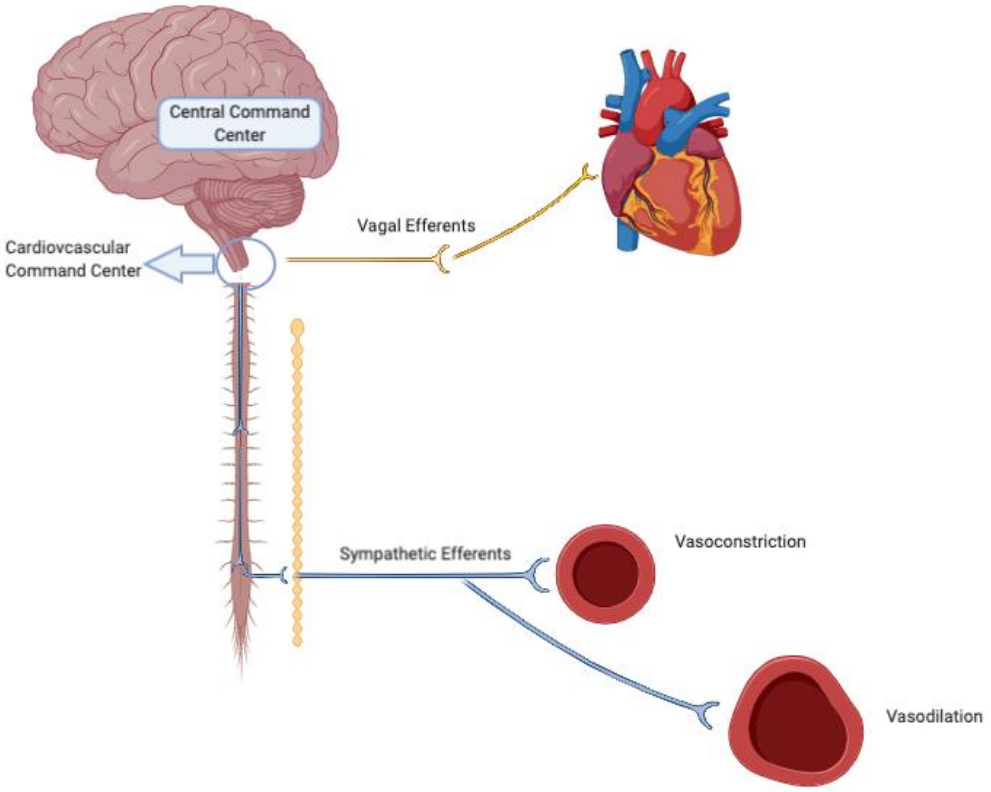
**Muscle Sympathetic Nerve Activity**

While there are certainly indices of sympathovagal balance that can be utilized to assess alterations in cardiovascular disease risk from inflammatory disease states, direct measures of sympathetic activity would allow a more precise understanding of the role of inflammation and oxidative stress in the development of cardiovascular disease with psoriasis. The study design

we have developed and describe in the next chapter is unique as it would be the first to directly assess sympathetic activation in the psoriatic population using microneurography. Thus, a brief discussion on the microneurographic technique is warranted.

Muscle sympathetic nerve activity (MSNA) is the impulse of the neural activity leading to vascular smooth muscle contraction or dilation. While autonomic function has been measured indirectly through blood pressure, heart rate variability, norepinephrine spill over, and skin conductance, microneurography allows for direct and reliable measurement of sympathetic nerve impulses (Yucha, 2000). Specifically, in order to assess sympathetic activity, postganglionic sympathetic efferent discharges leading to the vascular smooth muscle are recorded through a tungsten microelectrode (Figure 6).

**Figure 6**
*Neural cardiovascular control*




*Note.* Sympathetic activity routed from brain down to peripheral nerve junctions.

The first documented successful MSNA microneurography recording in human subjects was reported in 1968 by Vallo and Hagbarth (Mano, 2001; Vallbo et al., 1979) Since 1968, this procedure has been refined and is now employed throughout the world in laboratory settings.

The smooth muscle surrounding the vasculature regulates peripheral resistance to controls systemic blood pressure. MSNA is measured through microneurography, wherein a small microelectrode is inserted into the fascicles of a peripheral nerve (e.g., peroneal, radial, ulnar), innervating the blood vessels in the distal skeletal muscle. Continuous electrocardiogram (ECG) measurement is essential for determining a quality MSNA signals; as MSNA bursts are pulse synchronous. Pulse synchronicity along with other factors such as,

no response to stroking of the skin and increased pulse synchronous bursts during the Valsalva maneuver with a biphasic response, are indicative of nerve fibers specifically innervating skeletal muscle vasculature and not skin (Yucha, 2000).

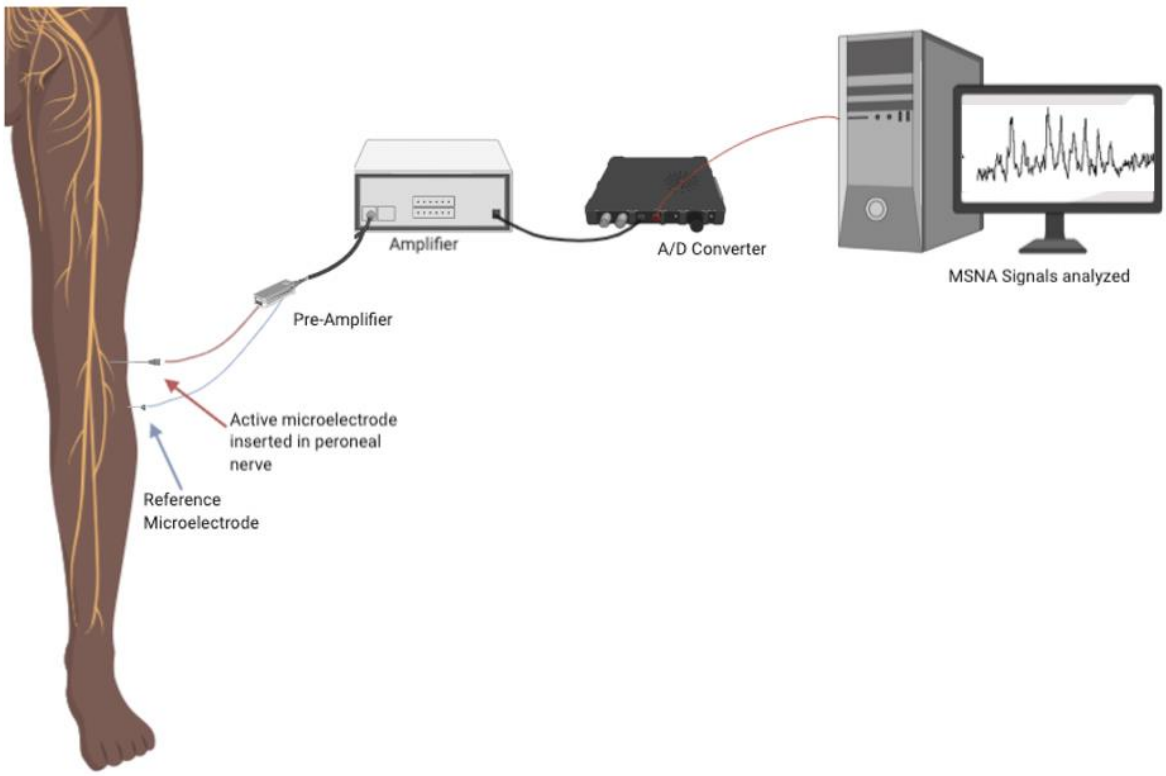
Oxidative stress and inflammation can subsequently increase sympathetic activity, and this has been observed regardless of how MSNA is quantified. MSNA typically is quantified and expressed as number of bursts per minute (burst frequency), the number of bursts per 100 heart beats (burst incidence), burst amplitude, and the area under the burst (total MSNA) (White et al., 2015). Determination of burst amplitude (and thus area) is achieved through a number of steps, First the mean voltage of a section with no bursts is calculated. This mean value is then reset to 0 arbitrary units (au), making any changes due to nerve traffic, and not back ground noise- a baseline (White et al., 2015). Once the baseline is established, burst height will also be normalized. Once the burst with the highest peak is located, the value will be assigned 100 au. This results in burst amplitude being relative to the largest burst size for that particular subject and test (White et al., 2015).

Two tungsten microelectrodes are utilized during microneurography. One is a reference/grounding electrode place superficially/subcutaneously, the second is an active electrode place into peripheral nerve of choice- often, the peroneal nerve near the fibular head. These recordings are directed through a preamplifier, then an amplifier. After they are amplified (50,000-100,000 times) they are full-wave rectified, band pass filtered (700-2000Hz), and integrated (White et al., 2015). The MSNA signal is then put through an A/D converter to then be analyzed and processed (Figure 7).

MSNA bursts are then analyzed based on sound, timing (i.e., relative to r-wave of ECG), and other visual characteristics. Anecdotally, the sound of efferent activity is similar to that of an ocean wave, where afferent activity is similar to the sound of gunshots.

**Figure 7**

*Microneurography*




*Note.* Microneurography laboratory set up.

To the best of our knowledge, MSNA has yet to be examined in subjects with psoriasis. However, based on increase in MSNA in other inflammatory diseases, we suspect sympathetic activity may also be elevated in individuals with psoriasis (Adlan et al., 2017; Grassi et al., 1998; Hering et al., 2007).

**Vascular Function**

Vascular function can be measured via a number of different protocols; two of the most common are pulse wave velocity (PWV) and endothelium-dependent flow-mediated dilation (FMD). PWV is one of the most universally recognized indications of arterial stiffness and is regularly implemented in identification of risk factors for cardiovascular disease (Pereira et al., 2015). In particular, cfPWV can predict the progression of increasing blood pressure and the development of hypertension in adults (Koivistoinen et al., 2018; Laurent et al., 2006; Mancia et al., 2013). Regardless of disease state, PWV is oftentimes used in clinical practice to assess risk of cardiovascular events and overall cardiovascular health.

**Figure 8**
*Flow mediated dilation*

*Note.* FMD laboratory set up.

FMD (Figure 8) is a measure of endothelial function. Endothelial dysfunction is characterized by the inability of the artery to vasodilate. Increased shear stress in a healthy artery stimulates NO production from the endothelial cell, which stimulates vasodilation. In a dysfunctional artery, this process is impeded by decreased NO bioavailability, causing the

vasculature to stay constricted, allowing damage to take place further exacerbating the vascular dysfunction. Flow mediated dilation is performed using a Doppler Ultrasound in B-Mode.

**Antioxidant Supplementation**

Vitamin C, vitamin E, and alpha lipoic acid are known to attenuate oxidative stress and improve vascular function in elderly persons, those with COPD, and those with heart failure (Ratchford et al., 2019). Based on the characteristic inflammation in the psoriatic population, it is reasonable to suspect that antioxidant supplementation may similarly improve vascular and autonomic function in these individuals; however, to date, the efficacy of antioxidant supplementation in psoriatic subjects is unclear.

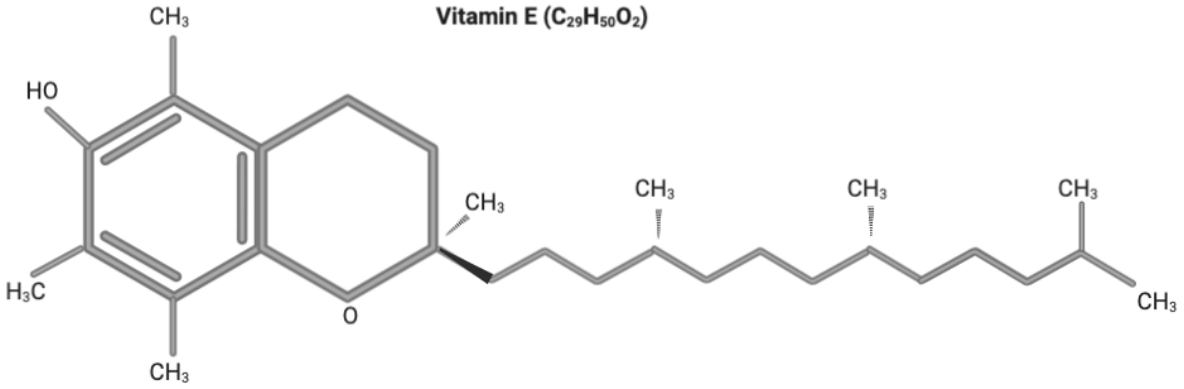
A substantial limitation in psoriatic research is the presence of comorbidities. In humans, psoriasis and comorbidities seem to be almost mutually inclusive. It is almost impossible to find subjects that are diagnosed with psoriasis but with no comorbidities. As such, there is still insufficient evidence from blinded and controlled studies on the efficacy of exogenous vitamin E, C, and alpha lipoic acid in the context of skin disorders. Further, because antioxidants are not a pharmaceutical drug, this also contributes to the lack of literature surrounding the topic (Thiele & Ekanayake-Mudiyanselage, 2007).

*Vitamin E*

The collective term “vitamin E” describes 8 fat soluble antioxidants. Alpha-tocopherol ($C_{29}H_{50}O_2$) is the orally bioavailable alpha form of the fat-soluble vitamin E (Figure 9). Upon oral administration, this antioxidant neutralizes free radicals, ultimately protecting tissues and vasculature from oxidative damage by incorporating itself into membranes, inhibiting lipid peroxidation and preventing protein oxidation. Ten-33% of alpha-tocopherol is able to be

absorbed in the small intestine, where bile and pancreatic enzymes are utilized for digestion. The exact mechanism(s) of action for most of Vitamin E's effects are still unknown (Enna, 1997).

***Figure 9***
*Vitamin E*




*Note.* Vitamin E chemical structure.

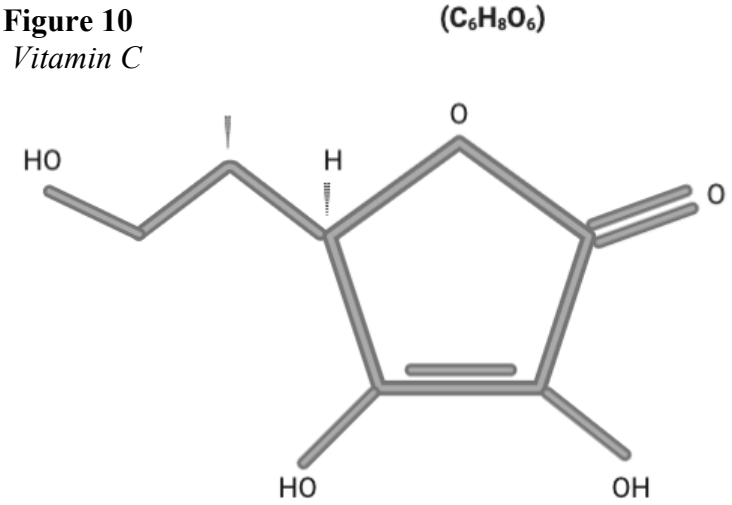
There have been a few promising studies surrounding vitamin E and skin disorders. Tsoureli-Nikita found that giving 400 IU/day of Vitamin E for 8 months caused near remission in subjects with dermatitis (Tsoureli-Nikita et al., 2002). Sander found that vitamin E slows cancer growth by inhibiting VEGF-mediated angiogenesis (Sander et al., 2003). With ROS-mediated VEGF- driven angiogenesis as a predominant feature of psoriasis, vitamin E supplementation has the potential to be beneficial.

*Vitamin C/Ascorbic Acid*

Ascorbic acid ($C_6H_8O_6$), or vitamin C, is a water-soluble vitamin with antioxidant properties; it must be obtained from the diet, as it cannot be synthesized or stored by humans (Enna, 1997)(Figure 11). Daily intake of vitamin C must be equal to or greater than the amount destroyed by oxidative processes or excretion. Those who are already under oxidative stress

and chronic systemic inflammation, require a higher dosage of vitamin C to maintain healthy values (Enna, 1997).

**Figure 10**
*Vitamin C*




*Note.* Vitamin C chemical

Vitamin C (Figure 10) is integral in differentiation of keratinocytes in healthy skin. A deficiency in Vitamin C is suspected to cause and/or aggravate certain skin diseases. This aggravation could be due in part to Vitamin C's essential roles in counteracting skin oxidation and modulating pathways for differentiation (Wang et al., 2018). A vitamin C deficiency is quite common, as vitamin C is only obtained through the diet; humans have no ability to synthesize vitamin C. However, a vitamin C deficiency is also easy to curb, and, accordingly it is probable that supplementation of vitamin C could increase the antioxidant properties of the skin, improving redox signaling balance.

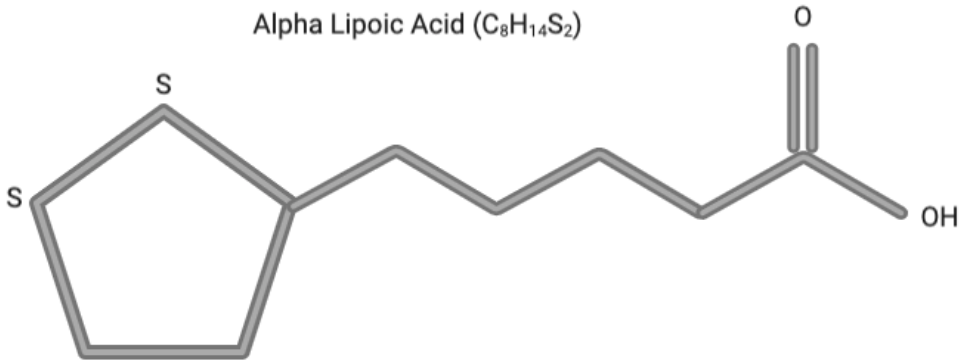
Furthermore, vitamin C has been shown to improve both cardiovagal (heart rate) and sympathetic (MSNA) baroreflex function (Bruno et al., 2012; Monahan et al., 2004; Nightingale et al., 2003). These findings indicate a link between oxidative stress and the baroreflex itself, which could explain, in part, the increased cardiovascular disease risk associated with oxidative stress. Vitamin C's efficacy is proposed to lie in its ability to increase

nitric oxide bioavailability, overall improving baroreflex sensitivity (Nightingale et al., 2003). Cardiovascular diseases are often chaperoned by baroreflex sensitivity issues where a chronic adrenergic activation is observed (La Rovere et al., 2008). If oxidative stress does indeed contribute mechanistically to cardiovagal and sympathetic baroreflex function, vitamin C supplementation could potentially dampen MSNA and increase baroreflex sensitivity, leading to reduction in sympathetic tone (Bruno et al., 2012; Monahan et al., 2004).

*Alpha Lipoic Acid:*

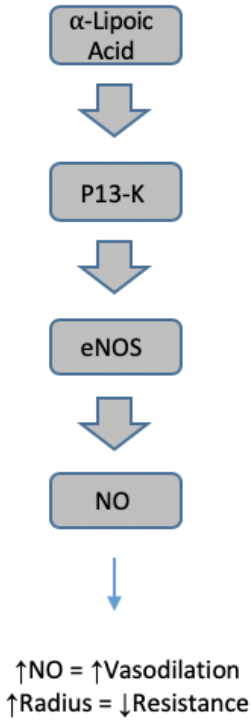
Alpha Lipoic Acid (ALA) ($C_8H_{14}O_2S_2$) is a potent antioxidant with anti-inflammatory properties (Figure 12). ALA is a dithiol compound that is synthesized from octanoic acid in the mitochondria (Badran et al., 2019). It is both lipid and water soluble, so it can be used in different environments within the cell.

**Figure 12**
*Alpha lipoic acid*




*Note.* Alpha lipoic acid structure.

**Figure 13**
*ALA pathway*




*Note.* Alpha Lipoic Acid (ALA) activating the P13-K pathway, allowing eNOS to synthesize nitric oxide (NO).

ALA has been shown to decrease oxidative stress effects in ischemia-reperfusion models, which is a suspected trigger for psoriasis (Herrling et al., 2003). Further, Heitzer et al found that ALA supplementation increased nitric oxide (NO) mediated vasodilation (Figure 9)-a measure of endothelial function-in patients with diabetes mellitus, a common comorbid condition among patients with psoriasis (Heitzer et al., 2001).

More importantly, ALA is known to have therapeutic effects on cardiovascular diseases by way of blood lipid modulating characteristics (e.g., hypertension) (Wollin & Jones, 2003). ALA has been shown to activate the P13-K pathway, one of many synthesizers of (NO), the most potent and well-studied endothelial-derived vasodilator (Ying et al., 2015). These compounding ill effects can lead to atherosclerosis, CVD, and/or severe cardiovascular events

(Boehncke et al., 2011; Hu & Lan, 2017; Wadley et al., 2013). Supplementing with ALA may help curb the psoriatic comorbidity of atherosclerosis by activating PI3-K/AKT pathway. Thus, oxidative stress and inflammation likely diminish NO bioavailability, which would subsequently limit the vasodilatory capacity.

## Conclusion

Overall, the presence of common inflammatory pathways, secretion of adipokines, insulin resistance, angiogenesis, and oxidative stress may explain the association between psoriasis and cardiovascular disease, and vascular events (Hu & Lan, 2017). There is a substantial amount of hypothesized inter-play specifically between systemic inflammation, reactive oxygen species, central adiposity, exercise tolerance, and MSNA in psoriasis. Antioxidant supplementation appears to be a potential low-cost means of ameliorating the elevated inflammation and oxidative stress, and subsequent impaired vascular and autonomic function, in this population. Future research examining vascular function and direct measures of muscle sympathetic nerve activity prior to and following an acute antioxidant supplementation will be important in providing scientists and clinicians a deeper understanding of the disease and its long-term implications, as well as potential avenues for more efficacious treatments in psoriatic patients.

## Chapter 3: Proposed Study Design and Anticipated Results

Due to the COVID-19 pandemic, a human subjects research study was unable to be conducted; however, the following is a study that has been designed to expand on the conclusions of the literature review and quantitatively assess the role of inflammation and oxidative stress in vascular and autonomic dysfunction in individuals with psoriasis.

The study described herein is designed to examine vascular function and muscle sympathetic nerve activity (MSNA) at rest and during a series of stressors, prior to and following a placebo or acute antioxidant supplementation intended to dampen systemic inflammation and oxidative stress, in diagnosed patients with psoriasis. We hypothesize that autonomic dysregulation and decrements in vascular function will be improved by an acute antioxidant supplementation. More specifically, the specific aims and hypotheses of this study are as follows:

**Specific Aim 1:** Identify the role of oxidative stress and inflammation on MSNA in patients with psoriasis via a placebo or acute antioxidant administration (Vitamin C 1000mg, Vitamin E 600IU, and Alpha Lipoic Acid 600mg) to reduce inflammation and oxidative stress.

- **Primary Hypothesis:** MSNA and inflammatory biomarkers (TNF-α and CRP) will be increased in patients with psoriasis when compared to normative values.
- **Secondary Hypothesis:** MSNA and inflammatory biomarkers (TNF-α and CRP) will decrease post-acute antioxidant supplementation and remain unchanged in the placebo group.

**Specific Aim 2:** Identify the role of oxidative stress and inflammation on vascular function in patients with psoriasis via acute antioxidant administration (Vitamin C 1000mg, Vitamin E 600IU, and Alpha Lipoic Acid 600mg) to reduce inflammation and oxidative stress.

- **Primary Hypothesis:** Patients with psoriasis will show lower arterial compliance, measured by pulse wave velocity, and endothelium-dependent flow mediated dilation when compared to normative values.
- **Secondary Hypothesis:** Arterial compliance and endothelium-dependent flow mediated dilation will increase post-acute antioxidant supplementation and remain unchanged in the placebo group.

**Delimitations**

Delimitations of this study design are as follows:

- Male and female subjects will be utilized
- Post-menopausal females will not be utilized
- Subjects on medication that have potential to influence the parasympathetic or sympathetic nervous system will be excluded
- The menstrual cycle will be controlled for by having subjects schedule visits around the onset of menstruation

**Limitations**

Limitations of this study design are as follows:

- Increased adiposity is known to influence (increase) MSNA. As most patients with psoriasis are overweight, this is an unavoidable confounding variable. Controls will be matched for body composition to the highest degree possible.
- MSNA signals may not be obtained on all subjects

**Study Design**

Subjects (n=20) between the ages of 18-45 years who have been diagnosed with psoriasis will be recruited from local dermatologists. Subjects will be randomly assigned to a control group (placebo; no antioxidant supplementation) or experimental group (antioxidant supplementation) by a random number generator. Subjects will come to the laboratory three times: Visit 1 will serve to determine eligibility, and Visits 2 and 3 will be experimental visits. Experimental testing will be carried out pre- and post- 14-day oral antioxidant or placebo supplementation. Subjects will be asked to arrive fasted and with ≥ 12 hours since last caffeinated or alcoholic beverage, as well as having abstained from vigorous exercise. Female subjects will have visits 2 and 3 conducted when they are menstruating.

**Visit 1 Testing**

Subjects will be taken through the informed consent process in accordance with the declaration of Helsinki. Eligibility for the study will be determined based on a health history questionnaire and a PASI score greater than 12, indicating moderate to severe psoriasis (Arnone et al., 2019; Colombo et al., 2008).

Additional inclusion criteria will be as follows:

- Age of eligibility (18-45 years) and a current diagnosis of psoriasis

- Psoriasis severity index score (PASI) >12

Exclusion criteria will be as follows (determined from Health History Questionnaire and Screening):

- Pregnant or trying to become pregnant
- Have been diagnosed with diabetes, cardiovascular disease, or peripheral vascular disease
- Partake in physical activity greater than two times per week
- Currently taking medications that may influence the parasympathetic or sympathetic nervous system
    - Sympathetic (adrenergic) agonists (e.g., phenylephrine),
    - 2) Sympatholytic drugs (adrenergic antagonists) (e.g., beta-blockers such as propranolol, alpha-blockers e.g., clonidine, Indoramin, Doxazosin),
    - 3) Parasympathetic agonists / Cholinergics (e.g., pilocarpine), and
    - 4) Parasympathetic antagonists / Anticholinergics (e.g., atropine, diphenhydramine (Benadryl), tricyclic antidepressants (Trazadone, Dresyl, Elavil, Tofranil).
- Prone to fainting/vasovagal syncope during needle like triggers (e.g. blood draws, shots)

Anthropometrics' and DEXA scan will then be completed. Females will take a pregnancy test (urine) prior to the DEXA scan. DEXA scanning uses radiation to obtain an image of the body- differentiating between muscle, bone, and fat.

This study will be a double-blind study, wherein subjects will be randomly assigned to a placebo or antioxidant supplementation group. Supplementation will take place over two-week

time period. Experimental Visits 2 and 3 will be scheduled around the menstrual cycle female subjects, where both will take place during the early follicular phase (i.e., menstruation). Therefore, menstruating female participants will wait to take their antioxidant or placebo supplementation until 2 weeks prior to their scheduled Visit 3.

**Visits 2 and 3 Testing**

*Biomarkers:* Subjects will have blood samples (24 mL) taken from the median cubital vein by a licensed and experienced research-team member for TNF-a, CRP, and total antioxidant capacity analysis. Blood draws will be completed at the beginning of Visit 2 (pre-intervention) and at the beginning of Visit 3 (post 14-day antioxidant supplementation) for biomarker indicators of inflammation levels, oxidative stress, and antioxidant capacity. Subjects will then undergo vascular and autonomic function testing.

*Vascular Function Testing:* Arterial stiffness will be assessed via carotid to femoral pulse wave velocity (automatic applanation tonometry). PWV measurements will be conducted using carotid-femoral (cfPWV) applanation tonometry (SphygmoCor XCEL, AtCor Medical, Sydney, New South Wales, Australia) with the subject in the supine position. The cfPWV will be calculated using the carotid and femoral pulses, the former by the tonometer, the latter by volumetric displacement of the femoral cuff. These two values will be indicative of the transit time (tt) of the blood bolus. Distances will be measured manually from the sternal notch (s) to thigh cuff (fC), the carotid artery (c), and the femoral artery (fT) to determine cfPWV:

$$cfPWV = \frac{d_{sfC} - d_{sc} - d_{fTfC}}{tt_{cfC} - k_1 - k_2 * d_{fTfC}}$$

where ($d_{fTfC}$) is the distance, $k_2$ is the time proportional to that distance. $K_1$ is another time correction used to adjust for the delay of the pressure transducer present in the femoral cuff (Butlin & Qasem, 2017).

Endothelium-dependent flow-mediated dilation (FMD) of the brachial artery will be measured using ultrasound and Qui Pu Cardiac Suite, allowing for conduit vessel endothelial dependent vasodilation as well as reactive hyperemia, an assessment of microvascular function. Flow mediated dilation will be performed using a Doppler Ultrasound in B-Mode utilizing the L4-12 probe (GE Logic 7 Ultrasound).

The probe will be placed between the biceps brachii and triceps on the medial aspect of the arm to access the brachial artery. Once a satisfactory view of the artery is achieved, 30 seconds of baseline blood flow will be recorded. Following a satisfactory baseline recording, the pneumatic tourniquet will be inflated to 250 mmHg for 5 minutes. 30 secs before the cuff is deflated recording will start to capture the ischemic response, with another 2 minutes of recording to follow.

*Autonomic function testing:* Subjects will have a short break and then be re-instrumented in the supine position on a tilt bed. MSNA will be recorded from the peroneal nerve at the fibular head by way of microneurography. LMX, Lidocaine, 4% Topical Anesthetic Cream will be used to numb participants during the microneurography procedures. Two small tungsten microelectrodes will be inserted, the reference microelectrode-needle inserted intradermally (~15°) and the active microelectrode-needle directly into the peroneal nerve. Beat-by-beat arterial blood pressure will be measured by finger photoplethysmography (Finapres) and heart rate will be monitored via 3-lead electrocardiogram. Respiratory rate will be measured by nasal cannula.

After acceptable efferent MSNA signals are obtained, autonomic function and hemodynamic data will be collected (BioPac) during a testing battery designed to stimulate changes in sympathetic activity:

- Spontaneous breathing for 6 mins (1 min baseline, 1 min recovery)
- Controlled breathing (12 breaths/min) for 6 mins (1 min baseline, 1 min recovery)
- Valsalva maneuver (40 mmHg for 15secs) (1 min baseline, 1 min recovery)
- Cold Pressor Test for 2 mins (1 min baseline, 3 min recovery)
- 3-min each of dynamic hand grip (DHG) at 30 and 45% maximal voluntary contraction (1 min baseline, 2 min recovery)
- Head-Up-Tilt (HUT) orthostatic challenges at 30° and 60° (1 min baseline, 2 min recovery)

MSNA, HR, BP, and respiratory rate will be continuously recorded throughout the autonomic testing.

*Blood Analysis:* Samples will be refrigerated, centrifuged, and analyzed via ELISA kits using immunometric assays.

*Data Analysis:* Signals will be sampled via data-acquisition system (BioPac) and analyzed using LabView software. MSNA will be quantified and expressed as number of bursts per minute (burst frequency), the number of bursts per 100 heart beats (burst incidence), and the area under the burst (total MSNA) (White et al., 2015). Blood pressure, heart rate, and respiratory rate will be averaged during spontaneous and controlled breathing. During tilt tests, the data will be averaged every min, including recovery.

*Statistical Analysis:* All statistical analyses will be performed using SPSS (Version 26, IBM, Armonk NY, US). Descriptive statistics will be used to describe characteristics of the groups. Data will be reported as mean ± standard deviation unless otherwise specified. A two-way repeated measures analysis of variance (ANOVA; condition x visit) will be utilized to examine the effect of antioxidant supplementation on measures of inflammatory markers, and vascular and autonomic function. For tests where there is a time component (i.e., cold pressor test, dynamic handgrip, head up tilt), three-way repeated measures ANOVA will be performed (condition x visit x time). Effect sizes will be calculated for the changes in dependent variables (e.g., inflammatory markers, MSNA, HR, BP, etc.) before and after antioxidant supplementation. Significance will be set at $p < 0.05$.

## Anticipated Results

Based on the findings of the literature review, the following section discusses the anticipated results of the research study that has been designed.

### Vascular function

Endothelial function, assessed by flow mediated dilation, has been shown to be largely intact in psoriatic subjects who have no traditional risk factors for cardiovascular disease (Martyn-Simmons et al., 2011). In turn, when not controlling for cardiovascular risk factors (i.e.; hypertension, waist circumference, resting heart rate) subjects with psoriasis do display compromised vascular function (Jensen et al., 2011). Furthermore, a consensus has yet to be reached on whether the immune modulating drugs used to treat psoriasis have an impact on cardiovascular risk and/or hence vascular function (Boehncke & Boehncke, 2011). Thus, we anticipate to see quite a range in this variable, which could be attributed to; predisposed cardiovascular risk factors (e.g.; increased adiposity, sedentary lifestyle, immune modulating drug use, pre or hypertension, etc.). Overall, we anticipate that the psoriatic subjects will display lower endothelial function when compared to normative values. Specifically, the subjects will have lower values of reactive hyperemia and absolute and relative (to the shear stimulus) percent changes in brachial artery diameter (i.e., absolute and relative FMD) compared with normative values.

Additionally, we expect that these assessments of vascular function in participants with psoriasis will show significant improvements following the two-week antioxidant supplementation due to increases in nitric oxide bioavailability, as supported by prior work in

heart failure patients (Bunsawat et al., 2020; Ratchford et al., 2019). We expect the placebo group will remain unchanged in this variable.

**Arterial Stiffness**

We anticipate that arterial stiffness, assessed as pulse wave velocity measured via arterial tonometry, will be augmented in the psoriatic subjects given prior work in this area (Liu et al., 2016; Sunbul et al., 2015). Children without cardiovascular risk factors who were diagnosed with psoriasis have significantly higher arterial stiffness compared with their healthy peers; indicating the systemic potential of the disease even in young vasculature (Şaylan Çevik et al., 2019). However, given the acute two-week supplementation, architectural changes to the arteries are unlikely in the experimental group. However, in different applications; like consumption of nicotine through cigarettes (Franzen et al., 2018) and exposure to black carbon (Provost et al., 2016), have been found to acutely increase arterial stiffness.

**Resting Sympathetic Activity and Hemodynamics**

We expect resting levels of MSNA, measured during the six-minute *spontaneous breathing* and *controlled breathing* test segments, to be elevated our subjects. Recent work in rheumatoid arthritis (RA) - which shares many similar inflammatory pathways and cardiovascular comorbidities with psoriasis (Stute & Koopmans, 2021) – shows elevated resting MSNA levels (33 ± 14) bursts/min in individuals with RA compared with age-matched healthy controls (20 ± 13) bursts/min (Peçanha et al., 2021). Furthermore, we anticipate based on prior work, that the subjects with psoriasis will have concomitantly higher resting arterial

systolic and diastolic blood pressures; as meta analyses found that psoriasis is associated with an increased risk for hypertension (Armesto et al., 2012; Duan et al., 2020).

Evidence showing the impact of antioxidant supplementation on MSNA is limited; however, an acute infusion of vitamin C has been shown to significantly lower MSNA in hypertensive subjects (Bruno et al., 2012). Given the efficacy of the antioxidant cocktail used in this study (Vitamins E, C, and alpha lipoic acid) on vascular function (Bunsawat et al., 2020; Ratchford et al., 2019), we expect to see concomitant reductions in resting sympathetic outflow in the experimental group and no change in the placebo group.

**Responses to the Valsalva Maneuver**

Baroreflex control of MSNA is reduced in the similar-to-psoriasis disease state of RA, as well as in hypertension and heart failure (Adlan et al., 2017; Peçanha et al., 2021). Furthermore, baroreflex sensitivity is also known to decrease with age (Gribbin et al., 1971; Toshiyoshi Matsukawa et al., 1998). As such, we anticipate that the subjects will have a suppressed, or less sensitive, sympathetic neural responses to the Valsalva maneuver when compared to previously collected healthy controls. While data on the impact of acute antioxidant supplementation on baroreflex sensitivity is limited, one animal study found that two-week alpha lipoic acid supplementation increased baroreflex sensitivity (Queiroz et al., 2012). The study described above would be the first to examine the impact of a short-term antioxidant supplementation on sympathetic baroreflex sensitivity in humans.

**Responses to the Cold Pressor Test**

The cold pressor test is a painful stimulus used to elicit increases in arterial pressure and MSNA (Fu et al., 2002; Victor et al., 1987; Yamamoto et al., 1992). Indeed, heightened blood pressure reactivity during the cold pressor test has been linked to hypertension and cardiovascular disease risk in adults (Wood et al., 1984; Zhao et al., 2015). We anticipate that those with psoriasis will have both elevated sympathetic neural (i.e.; MSNA) and hemodynamic (i.e., blood pressure) responses to the cold pressor test when compared to normative values. Following a 4-week dosing of vitamin E, responses to the cold pressor test (blood pressure, mean arterial pressure, forearm blood flow, and forearm vascular resistance) were decreased when compared to non-antioxidant conditions (Olatunji & Soladoye, 2008). Thus, we expect to see similar reductions in pressor responses during our intervention. However, sympathetic neural responses to the cold pressor test following antioxidant supplementation have yet to be measured. We expect MSNA reactivity (i.e., delta MSNA from baseline to peak of the cold pressor test) to be reduced in the experimental group and remain unchanged in the placebo group.

**Responses to Head Up Tilt**

In healthy individuals, MSNA plays a key role in mediating drops in blood pressure during orthostasis (Cui et al., 2011; Iwase et al., 1987). Hypertensive individuals are known to have exaggerated responses to head up tilt challenges due to heightened sympathetic outflow (Mark, 1990). As we are expecting augmentation of both pressures and sympathetic activity in those with psoriasis, we anticipate their responses to the orthostatic challenge will also be exaggerated when compared to normative values. Chronic, but not acute, dosage of vitamin E

has been shown to significantly decrease orthostatic tachycardia in healthy young men (Olatunji & Soladoye, 2008). As individuals with psoriasis may exhibit exaggerated responses at baseline, it is possible that these responses may become attenuated following even acute antioxidant administration.

## Conclusion

Individuals with psoriasis are more likely to die from heart attack, stroke, and cardiovascular disease when compared with their healthy counterparts. Much is unknown regarding the role of psoriasis in these observed cardiovascular comorbidities; the study described above has been designed to help elucidate the complex interactions between inflammation, oxidative stress, and vascular and autonomic function. The results from the study designed above will help guide scientists and clinicians towards more efficacious treatments for patients and a deeper understanding of the disease and its long-term implications.

## Chapter 4: National Psoriasis Foundation Grant Submission

The comprehensive literature review contained herein was utilized to prepare a grant for the National Psoriasis Foundation Early Career Research Grant mechanism, an award typically given to doctoral students and postdoctoral trainees. While the full grant application is not available for publication, below are the cover letter, specific aims, and notification from the granting agency.

## Chapter 5: *The Journal of Physiology* Journal Club Article Submission

Based largely upon the autoimmunity, autonomic function, and cardiovascular disease risk portions of the literature review, the following short review paper was submitted to and published in *The Journal of Physiology* as a Journal Club article in January 2021. This paper was written in response to / highlighting a *J Physiol* article by Pecanha et al. (Peçanha et al., 2021), which examined sympathetic and hemodynamic responses to exercise in rheumatoid arthritis. The submitted Journal Club article, entitled "Pushing the needle forward on the relationship between autoimmunity and autonomic dysfunction," (**https://doi.org/10.1113/JP281197**) can be found below.

## References


Abuabara, K., Azfar, R. S., Shin, D. B., Neimann, A. L., Troxel, A. B., & Gelfand, J. M. (2010, Sep). Cause-specific mortality in patients with severe psoriasis: a population-based cohort study in the U.K. *Br J Dermatol, 163*(3), 586-592. https://doi.org/10.1111/j.1365-2133.2010.09941.x

Adlan, A. M., Paton, J. F., Lip, G. Y., Kitas, G. D., & Fisher, J. P. (2017, Feb 1). Increased sympathetic nerve activity and reduced cardiac baroreflex sensitivity in rheumatoid arthritis. *J Physiol, 595*(3), 967-981. https://doi.org/10.1113/JP272944

Ahlehoff, O., Gislason, G. H., Jorgensen, C. H., Lindhardsen, J., Charlot, M., Olesen, J. B., Abildstrom, S. Z., Skov, L., Torp-Pedersen, C., & Hansen, P. R. (2012, Aug). Psoriasis and risk of atrial fibrillation and ischaemic stroke: a Danish Nationwide Cohort Study. *Eur Heart J, 33*(16), 2054-2064. https://doi.org/10.1093/eurheartj/ehr285

Alvarez, G. E., Beske, S. D., Ballard, T. P., & Davy, K. P. (2002, Nov 12). Sympathetic neural activation in visceral obesity. *Circulation, 106*(20), 2533-2536. https://doi.org/10.1161/01.cir.0000041244.79165.25

Anderson, E. A., Hoffman, R. P., Balon, T. W., Sinkey, C. A., & Mark, A. L. (1991). Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *The Journal of clinical investigation, 87(6)*(2246–2252).

Angum, F., Khan, T., Kaler, J., Siddiqui, L., & Hussain, A. (2020, May 13). The Prevalence of Autoimmune Disorders in Women: A Narrative Review. *Cureus, 12*(5), e8094. https://doi.org/10.7759/cureus.8094

Armesto, S., Coto-Segura, P., Osuna, C. G., Camblor, P. M., & Santos-Juanes, J. (2012, Jun). Psoriasis and hypertension: a case-control study. *J Eur Acad Dermatol Venereol, 26*(6), 785-788. https://doi.org/10.1111/j.1468-3083.2011.04108.x

Armstrong, A. W., Harskamp, C. T., & Armstrong, E. J. (2012, Dec 3). The association between psoriasis and obesity: a systematic review and meta-analysis of observational studies. *Nutr Diabetes, 2*, e54. https://doi.org/10.1038/nutd.2012.26

Armstrong, A. W., Voyles, S. V., Armstrong, E. J., Fuller, E. N., & Rutledge, J. C. (2011, Jul). Angiogenesis and oxidative stress: common mechanisms linking psoriasis with atherosclerosis. *J Dermatol Sci, 63*(1), 1-9. https://doi.org/10.1016/j.jdermsci.2011.04.007

Arnone, M., Takahashi, M. D. F., Carvalho, A. V. E., Bernardo, W. M., Bressan, A. L., Ramos, A. M. C., Terena, A. C., Souza, C. D. S., Nunes, D. H., Bortoletto, M. C. C., Oliveira, M., Neffa, J. M., Fieri, L. C., Azulay-Abulafia, L., Felix, P. A. O., Magalhaes, R. F., Romiti, R., & Jaime, T. J. (2019, Apr). Diagnostic and therapeutic guidelines for plaque psoriasis - Brazilian Society of Dermatology. *An Bras Dermatol, 94*(2 Suppl 1), 76-107. https://doi.org/10.1590/abd1806-4841.2019940211

Augustin, M., Radtke, M. A., Glaeske, G., Reich, K., Christophers, E., Schaefer, I., & Jacobi, A. (2015). Epidemiology and Comorbidity in Children with Psoriasis and Atopic Eczema. *Dermatology, 231*(1), 35-40. https://doi.org/10.1159/000381913

Augustin, M., Reich, K., Glaeske, G., Schaefer, I., & Radtke, M. (2010, Mar). Co-morbidity and age-related prevalence of psoriasis: Analysis of health insurance data in Germany. *Acta Derm Venereol, 90*(2), 147-151. https://doi.org/10.2340/00015555-0770

Ayala-Fontanez, N., Soler, D. C., & McCormick, T. S. (2016). Current knowledge on psoriasis and autoimmune diseases. *Psoriasis (Auckl), 6*, 7-32. https://doi.org/10.2147/ptt.S64950

Badran, M., Abuyassin, B., Golbidi, S., Ayas, N., & Laher, I. (2019). Alpha Lipoic Acid Improves Endothelial Function and Oxidative Stress in Mice Exposed to Chronic Intermittent Hypoxia. *Oxid Med Cell Longev, 2019*, 4093018. https://doi.org/10.1155/2019/4093018

Barretto, A. C., Santos, A. C., Munhoz, R., Rondon, M. U., Franco, F. G., Trombetta, I. C., Roveda, F., de Matos, L. N., Braga, A. M., Middlekauff, H. R., & Negrao, C. E. (2009, Jul 10). Increased muscle sympathetic nerve activity predicts mortality in heart failure patients. *Int J Cardiol, 135*(3), 302-307. https://doi.org/10.1016/j.ijcard.2008.03.056

Berne, C., Fagius, J., Pollare, T., & Hjemdahl, P. (1992, Sep). The sympathetic response to euglycaemic hyperinsulinaemia. Evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia, 35*(9), 873-879. https://doi.org/10.1007/bf00399935

Bhushan, M., McLaughlin, B., Weiss, J. B., & Griffiths, C. E. (1999, Dec). Levels of endothelial cell stimulating angiogenesis factor and vascular endothelial growth factor are elevated in psoriasis. *Br J Dermatol, 141*(6), 1054-1060. https://doi.org/10.1046/j.1365-2133.1999.03205.x

Boehncke, W. H., & Boehncke, S. (2011, Jun). Systemic antipsoriatic therapy may reverse endothelial dysfunction. *Br J Dermatol, 164*(6), 1397-1398; author reply 1398. https://doi.org/10.1111/j.1365-2133.2011.10250.x

Boehncke, W. H., Boehncke, S., Tobin, A. M., & Kirby, B. (2011, Apr). The 'psoriatic march': a concept of how severe psoriasis may drive cardiovascular comorbidity. *Exp Dermatol, 20*(4), 303-307. https://doi.org/10.1111/j.1600-0625.2011.01261.x

Brezinski, E. A., Dhillon, J. S., & Armstrong, A. W. (2015, Jun). Economic Burden of Psoriasis in the United States: A Systematic Review. *JAMA Dermatol, 151*(6), 651-658. https://doi.org/10.1001/jamadermatol.2014.3593

Briganti, S., & Picardo, M. (2003, Nov). Antioxidant activity, lipid peroxidation and skin diseases. What's new. *J Eur Acad Dermatol Venereol, 17*(6), 663-669. https://doi.org/10.1046/j.1468-3083.2003.00751.x

Brunner-La Rocca, H. P., Esler, M. D., Jennings, G. L., & Kaye, D. M. (2001, Jul). Effect of cardiac sympathetic nervous activity on mode of death in congestive heart failure. *Eur Heart J, 22*(13), 1136-1143. https://doi.org/10.1053/euhj.2000.2407

Bruno, R. M., Daghini, E., Ghiadoni, L., Sudano, I., Rugani, I., Varanini, M., Passino, C., Emdin, M., & Taddei, S. (2012, Aug). Effect of acute administration of vitamin C on muscle sympathetic

activity, cardiac sympathovagal balance, and baroreflex sensitivity in hypertensive patients. *Am J Clin Nutr, 96*(2), 302-308. https://doi.org/10.3945/ajcn.112.035022

Bunsawat, K., Ratchford, S. M., Alpenglow, J. K., Park, S. H., Jarrett, C. L., Stehlik, J., Drakos, S. G., Richardson, R. S., & Wray, D. W. (2020, Aug). Chronic antioxidant administration restores macrovascular function in patients with heart failure with reduced ejection fraction. *Exp Physiol, 105*(8), 1384-1395. https://doi.org/10.1113/ep088686

Burkhart C, M. D., Goldsmith L. (2011). Dermatological pharmacology. In *Goodman & Gilman's the pharmacological basis of therapeutics* (12th ed., pp. 1803-1832.). McGraw-Hill.

Butlin, M., & Qasem, A. (2017, Jan). Large Artery Stiffness Assessment Using SphygmoCor Technology. *Pulse (Basel), 4*(4), 180-192. https://doi.org/10.1159/000452448

Casey, D. P., Pierce, G. L., Howe, K. S., Mering, M. C., & Braith, R. W. (2007, Jul). Effect of resistance training on arterial wave reflection and brachial artery reactivity in normotensive postmenopausal women. *Eur J Appl Physiol, 100*(4), 403-408. https://doi.org/10.1007/s00421-007-0447-2

Colombel, J. F., Sandborn, W. J., Reinisch, W., Mantzaris, G. J., Kornbluth, A., Rachmilewitz, D., Lichtiger, S., D'Haens, G., Diamond, R. H., Broussard, D. L., Tang, K. L., van der Woude, C. J., Rutgeerts, P., & Group, S. S. (2010, Apr 15). Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med, 362*(15), 1383-1395. https://doi.org/10.1056/NEJMoa0904492

Colombo, G., Altomare, G., Peris, K., Martini, P., Quarta, G., Congedo, M., Costanzo, A., Di Cesare, A., Lapucci, E., & Chimenti, S. (2008, Apr). Moderate and severe plaque psoriasis: cost-of-illness study in Italy. *Ther Clin Risk Manag, 4*(2), 559-568. https://doi.org/10.2147/tcrm.s2740

Cui, J., Shibasaki, M., Low, D. A., Keller, D. M., Davis, S. L., & Crandall, C. G. (2011, Dec). Muscle sympathetic responses during orthostasis in heat-stressed individuals. *Clin Auton Res, 21*(6), 381-387. https://doi.org/10.1007/s10286-011-0126-6

Dhalla, N. S., Temsah, R. M., & Netticadan, T. (2000, Jun). Role of oxidative stress in cardiovascular diseases. *J Hypertens, 18*(6), 655-673. https://doi.org/10.1097/00004872-200018060-00002

Duan, X., Liu, J., Mu, Y., Liu, T., Chen, Y., Yu, R., Xiong, X., & Wu, T. (2020, Feb). A systematic review and meta-analysis of the association between psoriasis and hypertension with adjustment for covariates. *Medicine (Baltimore), 99*(9), e19303. https://doi.org/10.1097/md.0000000000019303

Duarte, G. V., Oliveira Mde, F., Cardoso, T. M., Follador, I., Silva, T. S., Cavalheiro, C. M., Nonato, W., & Carvalho, E. M. (2013, Feb). Association between obesity measured by different parameters and severity of psoriasis. *Int J Dermatol, 52*(2), 177-181. https://doi.org/10.1111/j.1365-4632.2011.05270.x

Duarte, G. V., & Silva, L. P. (2014, Sep-Oct). Correlation between psoriasis' severity and waist-to-height ratio. *An Bras Dermatol, 89*(5), 846-847. https://doi.org/10.1590/abd1806-4841.20142854

Eberle, F. C., Bruck, J., Holstein, J., Hirahara, K., & Ghoreschi, K. (2016). Recent advances in understanding psoriasis. *F1000Res, 5*. https://doi.org/10.12688/f1000research.7927.1

Enna, S. J. (1997, 1997/08/01). Goodman & Gilman's The Pharmacological Basis of Therapeutics Edited by Joel G. Hardman, Lee E. Limbird, Perry B. Molinoff, and Raymond W. Ruddon. McGraw-Hill, New York. 1996. xxi + 1905 pp. 21 × 26 cm. ISBN 0-07-026266-7. $89.00. *Journal of Medicinal Chemistry, 40*(16), 2657-2658. https://doi.org/10.1021/jm9703146

Fadel, P. J., Ogoh, S., Keller, D. M., & Raven, P. B. (2003, Nov). Recent insights into carotid baroreflex function in humans using the variable pressure neck chamber. *Exp Physiol, 88*(6), 671-680. https://doi.org/10.1113/eph8802650

Feldman, S. R. (2004, Jan). A quantitative definition of severe psoriasis for use in clinical trials. *J Dermatolog Treat, 15*(1), 27-29. https://doi.org/10.1080/09546630310019382

Flores, F., & Kerdel, F. A. (2000, Jul). Other novel immunosuppressants. *Dermatol Clin, 18*(3), 475-483, ix. https://doi.org/10.1016/s0733-8635(05)70195-9

Forstermann, U., & Munzel, T. (2006, Apr 4). Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation, 113*(13), 1708-1714. https://doi.org/10.1161/CIRCULATIONAHA.105.602532

Frampton, J., & Wagstaff, A. (2003). Alefacept. *Am J Clin Dermatol, 4*(4), 277-286; discussion 287. https://doi.org/10.2165/00128071-200304040-00006

Franzen, K. F., Willig, J., Cayo Talavera, S., Meusel, M., Sayk, F., Reppel, M., Dalhoff, K., Mortensen, K., & Droemann, D. (2018, Oct). E-cigarettes and cigarettes worsen peripheral and central hemodynamics as well as arterial stiffness: A randomized, double-blinded pilot study. *Vasc Med, 23*(5), 419-425. https://doi.org/10.1177/1358863x18779694

Fu, Q., Levine, B. D., Pawelczyk, J. A., Ertl, A. C., Diedrich, A., Cox, J. F., Zuckerman, J. H., Ray, C. A., Smith, M. L., Iwase, S., Saito, M., Sugiyama, Y., Mano, T., Zhang, R., Iwasaki, K., Lane, L. D., Buckey, J. C., Jr., Cooke, W. H., Robertson, R. M., Baisch, F. J., Blomqvist, C. G., Eckberg, D. L., Robertson, D., & Biaggioni, I. (2002, Oct 15). Cardiovascular and sympathetic neural responses to handgrip and cold pressor stimuli in humans before, during and after spaceflight. *J Physiol, 544*(2), 653-664. https://doi.org/10.1113/jphysiol.2002.025098

Fu, Q., & Ogoh, S. (2019, Nov). Sex differences in baroreflex function in health and disease. *J Physiol Sci, 69*(6), 851-859. https://doi.org/10.1007/s12576-019-00727-z

Garshick, M. S., Vaidean, G., Nikain, C. A., Chen, Y., Smilowitz, N. R., & Berger, J. S. (2019, Sep). Sex differences in the prevalence of vascular disease and risk factors in young hospitalized patients with psoriasis. *Int J Womens Dermatol, 5*(4), 251-255. https://doi.org/10.1016/j.ijwd.2019.05.003

Gelfand, J. M., Neimann, A. L., Shin, D. B., Wang, X., Margolis, D. J., & Troxel, A. B. (2006, Oct 11). Risk of myocardial infarction in patients with psoriasis. *JAMA, 296*(14), 1735-1741. https://doi.org/10.1001/jama.296.14.1735

Gelfand, J. M., Troxel, A. B., Lewis, J. D., Kurd, S. K., Shin, D. B., Wang, X., Margolis, D. J., & Strom, B. L. (2007, Dec). The risk of mortality in patients with psoriasis: results from a

population-based study. *Arch Dermatol, 143*(12), 1493-1499. https://doi.org/10.1001/archderm.143.12.1493

Georgiopoulos, G., Chrysohoou, C., Vogiatzi, G., Magkas, N., Bournelis, I., Bampali, S., Gruson, D., & Tousoulis, D. (2017). Vitamins in Heart Failure: Friend or Enemy? *Curr Pharm Des, 23*(25), 3731-3742. https://doi.org/10.2174/1381612823666170321094711

Gisondi, P., Tessari, G., Conti, A., Piaserico, S., Schianchi, S., Peserico, A., Giannetti, A., & Girolomoni, G. (2007, Jul). Prevalence of metabolic syndrome in patients with psoriasis: a hospital-based case-control study. *Br J Dermatol, 157*(1), 68-73. https://doi.org/10.1111/j.1365-2133.2007.07986.x

Grassi, G., Colombo, M., Seravalle, G., Spaziani, D., & Mancia, G. (1998, Jan). Dissociation between muscle and skin sympathetic nerve activity in essential hypertension, obesity, and congestive heart failure. *Hypertension, 31*(1), 64-67. https://doi.org/10.1161/01.hyp.31.1.64

Grassi, G., Dell'Oro, R., Facchini, A., Quarti Trevano, F., Bolla, G. B., & Mancia, G. (2004, Dec). Effect of central and peripheral body fat distribution on sympathetic and baroreflex function in obese normotensives. *J Hypertens, 22*(12), 2363-2369. https://doi.org/10.1097/00004872-200412000-00019

Grassi, G., Mark, A., & Esler, M. (2015, Mar 13). The sympathetic nervous system alterations in human hypertension. *Circ Res, 116*(6), 976-990. https://doi.org/10.1161/circresaha.116.303604

Grassi, G., Seravalle, G., Cattaneo, B. M., Bolla, G. B., Lanfranchi, A., Colombo, M., Giannattasio, C., Brunani, A., Cavagnini, F., & Mancia, G. (1995, Apr). Sympathetic activation in obese normotensive subjects. *Hypertension, 25*(4 Pt 1), 560-563. https://doi.org/10.1161/01.hyp.25.4.560

Gribbin, B., Pickering, T. G., Sleight, P., & Peto, R. (1971, Oct). Effect of age and high blood pressure on baroreflex sensitivity in man. *Circ Res, 29*(4), 424-431. https://doi.org/10.1161/01.res.29.4.424

Griffiths, C. E., & Barker, J. N. (2007, Jul 21). Pathogenesis and clinical features of psoriasis. *Lancet, 370*(9583), 263-271. https://doi.org/10.1016/s0140-6736(07)61128-3

Gudjonsson, J. E., Johnston, A., Sigmundsdottir, H., & Valdimarsson, H. (2004, Jan). Immunopathogenic mechanisms in psoriasis. *Clin Exp Immunol, 135*(1), 1-8. https://doi.org/10.1111/j.1365-2249.2004.02310.x

Hägg, D., Sundström, A., Eriksson, M., & Schmitt-Egenolf, M. (2017, Aug). Severity of Psoriasis Differs Between Men and Women: A Study of the Clinical Outcome Measure Psoriasis Area and Severity Index (PASI) in 5438 Swedish Register Patients. *Am J Clin Dermatol, 18*(4), 583-590. https://doi.org/10.1007/s40257-017-0274-0

Hart, E. C., Charkoudian, N., Joyner, M. J., Barnes, J. N., Curry, T. B., & Casey, D. P. (2013, Sep). Relationship between sympathetic nerve activity and aortic wave reflection characteristics in postmenopausal women. *Menopause, 20*(9), 967-972. https://doi.org/10.1097/GME.0b013e3182843b59

Hayano, J., & Yuda, E. (2019, Mar 13). Pitfalls of assessment of autonomic function by heart rate variability. *J Physiol Anthropol, 38*(1), 3. https://doi.org/10.1186/s40101-019-0193-2

Heath, M. S., Sahni, D. R., Curry, Z. A., & Feldman, S. R. (2018, Sep). Pharmacokinetics of tazarotene and acitretin in psoriasis. *Expert Opin Drug Metab Toxicol, 14*(9), 919-927. https://doi.org/10.1080/17425255.2018.1515198

Heitzer, T., Finckh, B., Albers, S., Krohn, K., Kohlschutter, A., & Meinertz, T. (2001, Jul 1). Beneficial effects of alpha-lipoic acid and ascorbic acid on endothelium-dependent, nitric oxide-mediated vasodilation in diabetic patients: relation to parameters of oxidative stress. *Free Radic Biol Med, 31*(1), 53-61. https://doi.org/10.1016/s0891-5849(01)00551-2

Hering, D., Zdrojewski, Z., Krol, E., Kara, T., Kucharska, W., Somers, V. K., Rutkowski, B., & Narkiewicz, K. (2007, Jan). Tonic chemoreflex activation contributes to the elevated muscle sympathetic nerve activity in patients with chronic renal failure. *J Hypertens, 25*(1), 157-161. https://doi.org/10.1097/HJH.0b013e3280102d92

Herrling, T., Fuchs, J., Rehberg, J., & Groth, N. (2003, Jul 1). UV-induced free radicals in the skin detected by ESR spectroscopy and imaging using nitroxides. *Free Radic Biol Med, 35*(1), 59-67. https://doi.org/10.1016/s0891-5849(03)00241-7

Heydendael, V. M., Spuls, P. I., Opmeer, B. C., de Borgie, C. A., Reitsma, J. B., Goldschmidt, W. F., Bossuyt, P. M., Bos, J. D., & de Rie, M. A. (2003, Aug 14). Methotrexate versus cyclosporine in moderate-to-severe chronic plaque psoriasis. *N Engl J Med, 349*(7), 658-665. https://doi.org/10.1056/NEJMoa021359

Holwerda, S. W., Luehrs, R. E., DuBose, L., Collins, M. T., Wooldridge, N. A., Stroud, A. K., Fadel, P. J., Abboud, F. M., & Pierce, G. L. (2019, May). Elevated Muscle Sympathetic Nerve Activity Contributes to Central Artery Stiffness in Young and Middle-Age/Older Adults. *Hypertension, 73*(5), 1025-1035. https://doi.org/10.1161/hypertensionaha.118.12462

Hu, S. C., & Lan, C. E. (2017, Oct 21). Psoriasis and Cardiovascular Comorbidities: Focusing on Severe Vascular Events, Cardiovascular Risk Factors and Implications for Treatment. *Int J Mol Sci, 18*(10). https://doi.org/10.3390/ijms18102211

Hu, Y., Chen, Z., Gong, Y., & Shi, Y. (2018, Mar). A Review of Switching Biologic Agents in the Treatment of Moderate-to-Severe Plaque Psoriasis. *Clin Drug Investig, 38*(3), 191-199. https://doi.org/10.1007/s40261-017-0603-3

Hubert, H. B., Feinleib, M., McNamara, P. M., & Castelli, W. P. (1983, May). Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation, 67*(5), 968-977. https://doi.org/10.1161/01.cir.67.5.968

Huggett, R. J., Burns, J., Mackintosh, A. F., & Mary, D. A. (2004, Dec). Sympathetic neural activation in nondiabetic metabolic syndrome and its further augmentation by hypertension. *Hypertension, 44*(6), 847-852. https://doi.org/10.1161/01.HYP.0000147893.08533.d8

Huggett, R. J., Scott, E. M., Gilbey, S. G., Stoker, J. B., Mackintosh, A. F., & Mary, D. A. (2003, Dec 23). Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. *Circulation, 108*(25), 3097-3101. https://doi.org/10.1161/01.Cir.0000103123.66264.Fe

Inaba, Y., Chen, J. A., & Bergmann, S. R. (2010, Aug). Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging, 26*(6), 631-640. https://doi.org/10.1007/s10554-010-9616-1

Iwase, S., Mano, T., & Saito, M. (1987, Feb). Effects of graded head-up tilting on muscle sympathetic activities in man. *Physiologist, 30*(1 Suppl), S62-63.

Jensen, P. R., Zachariae, C., Hansen, P., & Skov, L. (2011, Sep). Normal endothelial function in patients with mild-to-moderate psoriasis: a case-control study. *Acta Derm Venereol, 91*(5), 516-520. https://doi.org/10.2340/00015555-1110

Kaye, J. A., Li, L., & Jick, S. S. (2008, Sep). Incidence of risk factors for myocardial infarction and other vascular diseases in patients with psoriasis. *Br J Dermatol, 159*(4), 895-902. https://doi.org/10.1111/j.1365-2133.2008.08707.x

Koivistoinen, T., Lyytikainen, L. P., Aatola, H., Luukkaala, T., Juonala, M., Viikari, J., Lehtimaki, T., Raitakari, O. T., Kahonen, M., & Hutri-Kahonen, N. (2018, Mar). Pulse Wave Velocity Predicts the Progression of Blood Pressure and Development of Hypertension in Young Adults. *Hypertension, 71*(3), 451-456. https://doi.org/10.1161/HYPERTENSIONAHA.117.10368

Krueger, G., & Callis, K. (2004, Feb). Potential of tumor necrosis factor inhibitors in psoriasis and psoriatic arthritis. *Arch Dermatol, 140*(2), 218-225. https://doi.org/10.1001/archderm.140.2.218

La Rovere, M. T., Pinna, G. D., & Raczak, G. (2008, Apr). Baroreflex sensitivity: measurement and clinical implications. *Ann Noninvasive Electrocardiol, 13*(2), 191-207. https://doi.org/10.1111/j.1542-474X.2008.00219.x

Lago, R., Gomez, R., Lago, F., Gomez-Reino, J., & Gualillo, O. (2008, Mar-Apr). Leptin beyond body weight regulation--current concepts concerning its role in immune function and inflammation. *Cell Immunol, 252*(1-2), 139-145. https://doi.org/10.1016/j.cellimm.2007.09.004

Lambert, E., Sari, C. I., Dawood, T., Nguyen, J., McGrane, M., Eikelis, N., Chopra, R., Wong, C., Chatzivlastou, K., Head, G., Straznicky, N., Esler, M., Schlaich, M., & Lambert, G. (2010, Sep). Sympathetic nervous system activity is associated with obesity-induced subclinical organ damage in young adults. *Hypertension, 56*(3), 351-358. https://doi.org/10.1161/hypertensionaha.110.155663

Lambert, E., Straznicky, N., Schlaich, M., Esler, M., Dawood, T., Hotchkin, E., & Lambert, G. (2007, Nov). Differing pattern of sympathoexcitation in normal-weight and obesity-related hypertension. *Hypertension, 50*(5), 862-868. https://doi.org/10.1161/hypertensionaha.107.094649

Landsberg, L. (1986, Dec). Diet, obesity and hypertension: an hypothesis involving insulin, the sympathetic nervous system, and adaptive thermogenesis. *Q J Med, 61*(236), 1081-1090.

Laurent, S., Cockcroft, J., Van Bortel, L., Boutouyrie, P., Giannattasio, C., Hayoz, D., Pannier, B., Vlachopoulos, C., Wilkinson, I., Struijker-Boudier, H., & European Network for Non-invasive Investigation of Large, A. (2006, Nov). Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J, 27*(21), 2588-2605. https://doi.org/10.1093/eurheartj/ehl254

Lin, X., & Huang, T. (2016, Jun). Oxidative stress in psoriasis and potential therapeutic use of antioxidants. *Free Radic Res, 50*(6), 585-595. https://doi.org/10.3109/10715762.2016.1162301

Liu, J. H., Chen, Y., Zhen, Z., Yeung, C. K., Chan, J., Chan, H. H., Tse, H. F., & Yiu, K. H. (2016, Aug). Relation between endothelial progenitor cells and arterial stiffness in patients with psoriasis. *J Dermatol, 43*(8), 888-893. https://doi.org/10.1111/1346-8138.13235

Lowes, M. A., Suarez-Farinas, M., & Krueger, J. G. (2014). Immunology of psoriasis. *Annu Rev Immunol, 32*, 227-255. https://doi.org/10.1146/annurev-immunol-032713-120225

Ludwig, R. J., Herzog, C., Rostock, A., Ochsendorf, F. R., Zollner, T. M., Thaci, D., Kaufmann, R., Vogl, T. J., & Boehncke, W. H. (2007, Feb). Psoriasis: a possible risk factor for development of coronary artery calcification. *Br J Dermatol, 156*(2), 271-276. https://doi.org/10.1111/j.1365-2133.2006.07562.x

Macefield, V. G. (2013). Sympathetic microneurography. *Handb Clin Neurol, 117*, 353-364. https://doi.org/10.1016/B978-0-444-53491-0.00028-6

Major, E. O. (2010). Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies. *Annu Rev Med, 61*, 35-47. https://doi.org/10.1146/annurev.med.080708.082655

Malpas, S. C. (2010, Apr). Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiol Rev, 90*(2), 513-557. https://doi.org/10.1152/physrev.00007.2009

Mancia, G., Fagard, R., Narkiewicz, K., Redon, J., Zanchetti, A., Bohm, M., Christiaens, T., Cifkova, R., De Backer, G., Dominiczak, A., Galderisi, M., Grobbee, D. E., Jaarsma, T., Kirchhof, P., Kjeldsen, S. E., Laurent, S., Manolis, A. J., Nilsson, P. M., Ruilope, L. M., Schmieder, R. E., Sirnes, P. A., Sleight, P., Viigimaa, M., Waeber, B., Zannad, F., & Task Force, M. (2013, Jul). 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens, 31*(7), 1281-1357. https://doi.org/10.1097/01.hjh.0000431740.32696.cc

Mano, T. (2001, Oct). Muscle sympathetic nerve activity in blood pressure control against gravitational stress. *J Cardiovasc Pharmacol, 38 Suppl 1*, S7-11. https://doi.org/10.1097/00005344-200110001-00003

Mark, A. L. (1990, Dec). Regulation of sympathetic nerve activity in mild human hypertension. *J Hypertens Suppl, 8*(7), S67-75.

Martyn-Simmons, C. L., Ranawaka, R. R., Chowienczyk, P., Crook, M. A., Marber, M. S., Smith, C. H., & Barker, J. N. (2011, Jan). A prospective case-controlled cohort study of endothelial function in patients with moderate to severe psoriasis. *Br J Dermatol, 164*(1), 26-32. https://doi.org/10.1111/j.1365-2133.2010.10031.x

Matsukawa, T., Sugiyama, Y., Watanabe, T., Kobayashi, F., & Mano, T. (1998a, 1998/11/10/). Baroreflex control of muscle sympathetic nerve activity is attenuated in the elderly. *Journal of the Autonomic Nervous System, 73*(2), 182-185. https://doi.org/https://doi.org/10.1016/S0165-1838(98)00128-3

Matsukawa, T., Sugiyama, Y., Watanabe, T., Kobayashi, F., & Mano, T. (1998b, Nov). Gender difference in age-related changes in muscle sympathetic nerve activity in healthy subjects. *Am J Physiol, 275*(5), R1600-1604. https://doi.org/10.1152/ajpregu.1998.275.5.R1600

Mehta, N. N., Yu, Y., Pinnelas, R., Krishnamoorthy, P., Shin, D. B., Troxel, A. B., & Gelfand, J. M. (2011, Aug). Attributable risk estimate of severe psoriasis on major cardiovascular events. *Am J Med, 124*(8), 775 e771-776. https://doi.org/10.1016/j.amjmed.2011.03.028

Meijer, K., de Vries, M., Al-Lahham, S., Bruinenberg, M., Weening, D., Dijkstra, M., Kloosterhuis, N., van der Leij, R. J., van der Want, H., Kroesen, B. J., Vonk, R., & Rezaee, F. (2011, Mar 23). Human primary adipocytes exhibit immune cell function: adipocytes prime inflammation independent of macrophages. *PLoS One, 6*(3), e17154. https://doi.org/10.1371/journal.pone.0017154

Miller, I. M., Skaaby, T., Ellervik, C., & Jemec, G. B. (2013, Dec). Quantifying cardiovascular disease risk factors in patients with psoriasis: a meta-analysis. *Br J Dermatol, 169*(6), 1180-1187. https://doi.org/10.1111/bjd.12490

Monahan, K. D., Eskurza, I., & Seals, D. R. (2004, Jun). Ascorbic acid increases cardiovagal baroreflex sensitivity in healthy older men. *Am J Physiol Heart Circ Physiol, 286*(6), H2113-2117. https://doi.org/10.1152/ajpheart.01054.2003

Moreira, H. G., Lage, R. L., Martinez, D. G., Ferreira-Santos, L., Rondon, M., Negrao, C. E., & Nicolau, J. C. (2017, May 1). Sympathetic nervous activity in patients with acute coronary syndrome: a comparative study of inflammatory biomarkers. *Clin Sci (Lond), 131*(9), 883-895. https://doi.org/10.1042/CS20170049

Myers, J., Prakash, M., Froelicher, V., Do, D., Partington, S., & Atwood, J. E. (2002, Mar 14). Exercise capacity and mortality among men referred for exercise testing. *N Engl J Med, 346*(11), 793-801. https://doi.org/10.1056/NEJMoa011858

Mysliwiec, H., Baran, A., Harasim-Symbor, E., Mysliwiec, P., Milewska, A. J., Chabowski, A., & Flisiak, I. (2017, Jul). Serum fatty acid profile in psoriasis and its comorbidity. *Arch Dermatol Res, 309*(5), 371-380. https://doi.org/10.1007/s00403-017-1748-x

Nestle, F. O., Kaplan, D. H., & Barker, J. (2009, Jul 30). Psoriasis. *N Engl J Med, 361*(5), 496-509. https://doi.org/10.1056/NEJMra0804595

Nightingale, A. K., Blackman, D. J., Field, R., Glover, N. J., Pegge, N., Mumford, C., Schmitt, M., Ellis, G. R., Morris-Thurgood, J. A., & Frenneaux, M. P. (2003, May). Role of nitric oxide and oxidative stress in baroreceptor dysfunction in patients with chronic heart failure. *Clin Sci (Lond), 104*(5), 529-535. https://doi.org/10.1042/CS20020334

Niki, E. (2014, May-Jun). Antioxidants: basic principles, emerging concepts, and problems. *Biomed J, 37*(3), 106-111. https://doi.org/10.4103/2319-4170.128727

Olatunji, L. A., & Soladoye, A. O. (2008, Dec). Effects of chronic administration of vitamin E on haemodynamic responses to postural stress or cold pressor test in apparently healthy young men. *Niger Postgrad Med J, 15*(4), 225-228.

Pearce, D. J., Lucas, J., Wood, B., Chen, J., Balkrishnan, R., & Feldman, S. R. (2006). Death from psoriasis: representative US data. *J Dermatolog Treat, 17*(5), 302-303. https://doi.org/10.1080/09546630600825067

Peçanha, T., Meireles, K., Pinto, A. J., Rezende, D. A. N., Iraha, A. Y., Mazzolani, B. C., Smaira, F. I., Sales, A. R. K., Bonfiglioli, K., Sá-Pinto, A. L., Lima, F. R., Irigoyen, M. C., Gualano, B., & Roschel, H. (2021, Feb). Increased sympathetic and haemodynamic responses to exercise and muscle metaboreflex activation in post-menopausal women with rheumatoid arthritis. *J Physiol, 599*(3), 927-941. https://doi.org/10.1113/jp280892

Pereira, T., Correia, C., & Cardoso, J. (2015). Novel Methods for Pulse Wave Velocity Measurement. *J Med Biol Eng, 35*(5), 555-565. https://doi.org/10.1007/s40846-015-0086-8

Popov, I., & Lewin, G. (1991, Jul). A deficient function of the antioxidative system of the organism as an aetiopathogenetic factor in psoriasis. *Med Hypotheses, 35*(3), 229-236. https://doi.org/10.1016/0306-9877(91)90238-t

Provost, E. B., Louwies, T., Cox, B., Op 't Roodt, J., Solmi, F., Dons, E., Int Panis, L., De Boever, P., & Nawrot, T. S. (2016, Mar). Short-term fluctuations in personal black carbon exposure are associated with rapid changes in carotid arterial stiffening. *Environ Int, 88*, 228-234. https://doi.org/10.1016/j.envint.2015.12.023

Puig, L. (2011, Sep). Obesity and psoriasis: body weight and body mass index influence the response to biological treatment. *J Eur Acad Dermatol Venereol, 25*(9), 1007-1011. https://doi.org/10.1111/j.1468-3083.2011.04065.x

Queiroz, T. M., Guimarães, D. D., Mendes-Junior, L. G., & Braga, V. A. (2012, Nov 9). α-lipoic acid reduces hypertension and increases baroreflex sensitivity in renovascular hypertensive rats. *Molecules, 17*(11), 13357-13367. https://doi.org/10.3390/molecules171113357

Rahmouni, K., Correia, M. L., Haynes, W. G., & Mark, A. L. (2005, Jan). Obesity-associated hypertension: new insights into mechanisms. *Hypertension, 45*(1), 9-14. https://doi.org/10.1161/01.HYP.0000151325.83008.b4

Ratchford, S. M., Clifton, H. L., Gifford, J. R., La Salle, D. T., Thurston, T. S., Bunsawat, K., Alpenglow, J. K., Richardson, R. S., Wright, J. B., Ryan, J. J., & Wray, D. W. (2019, Sep 4). Impact of Acute Antioxidant Administration on Inflammation and Vascular Function in Heart Failure with Preserved Ejection Fraction. *Am J Physiol Regul Integr Comp Physiol*. https://doi.org/10.1152/ajpregu.00184.2019

Reczek, C. R., & Chandel, N. S. (2015, Apr). ROS-dependent signal transduction. *Curr Opin Cell Biol, 33*, 8-13. https://doi.org/10.1016/j.ceb.2014.09.010

Reich, K. (2012, Mar). The concept of psoriasis as a systemic inflammation: implications for disease management. *J Eur Acad Dermatol Venereol, 26 Suppl 2*, 3-11. https://doi.org/10.1111/j.1468-3083.2011.04410.x

Robinson, A., Van Voorhees, A. S., Hsu, S., Korman, N. J., Lebwohl, M. G., Bebo, B. F., Jr., & Kalb, R. E. (2012, Aug). Treatment of pustular psoriasis: from the Medical Board of the National Psoriasis Foundation. *J Am Acad Dermatol, 67*(2), 279-288. https://doi.org/10.1016/j.jaad.2011.01.032

Rosado-Perez, J., & Mendoza-Nunez, V. M. (2018, Apr-Jun). Relationship Between Aerobic Capacity With Oxidative Stress and Inflammation Biomarkers in the Blood of Older Mexican Urban-Dwelling Population. *Dose Response, 16*(2), 1559325818773000. https://doi.org/10.1177/1559325818773000

Rosenbach, M., Hsu, S., Korman, N. J., Lebwohl, M. G., Young, M., Bebo, B. F., Jr., & Van Voorhees, A. S. (2010, Apr). Treatment of erythrodermic psoriasis: from the medical board of the National Psoriasis Foundation. *J Am Acad Dermatol, 62*(4), 655-662. https://doi.org/10.1016/j.jaad.2009.05.048

Ross, R. (1999, Jan 14). Atherosclerosis--an inflammatory disease. *N Engl J Med, 340*(2), 115-126. https://doi.org/10.1056/NEJM199901143400207

Sander, C. S., Hamm, F., Elsner, P., & Thiele, J. J. (2003, May). Oxidative stress in malignant melanoma and non-melanoma skin cancer. *Br J Dermatol, 148*(5), 913-922. https://doi.org/10.1046/j.1365-2133.2003.05303.x

Sanyal, A. J., Chalasani, N., Kowdley, K. V., McCullough, A., Diehl, A. M., Bass, N. M., Neuschwander-Tetri, B. A., Lavine, J. E., Tonascia, J., Unalp, A., Van Natta, M., Clark, J., Brunt, E. M., Kleiner, D. E., Hoofnagle, J. H., & Robuck, P. R. (2010, May 6). Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med, 362*(18), 1675-1685. https://doi.org/10.1056/NEJMoa0907929

Şaylan Çevik, B., Akalın, F., Erolu, E., Gençosmanoğlu, S., & Ergun, T. (2019, Apr 18). Assessment of cardiac and vessel functions in childhood psoriasis. *Turk J Med Sci, 49*(2), 617-623. https://doi.org/10.3906/sag-1812-139

Schieber, M., & Chandel, N. S. (2014, May 19). ROS function in redox signaling and oxidative stress. *Curr Biol, 24*(10), R453-462. https://doi.org/10.1016/j.cub.2014.03.034

Schmitt, J., & Wozel, G. (2005). The psoriasis area and severity index is the adequate criterion to define severity in chronic plaque-type psoriasis. *Dermatology, 210*(3), 194-199. https://doi.org/10.1159/000083509

Sinha, N., & Dabla, P. K. (2015). Oxidative stress and antioxidants in hypertension-a current review. *Curr Hypertens Rev, 11*(2), 132-142. https://doi.org/10.2174/1573402111666150529130922

Sivenius, K., Niskanen, L., Laakso, M., & Uusitupa, M. (2003, Aug). A deletion in the alpha2B-adrenergic receptor gene and autonomic nervous function in central obesity. *Obes Res, 11*(8), 962-970. https://doi.org/10.1038/oby.2003.133

Smith, M. M., & Minson, C. T. (2012, Apr 15). Obesity and adipokines: effects on sympathetic overactivity. *J Physiol, 590*(8), 1787-1801. https://doi.org/10.1113/jphysiol.2011.221036

Stute, N. L., & Koopmans, P. J. (2021, Feb). Pushing the needle forward on the relationship between autoimmunity and autonomic dysfunction. *J Physiol, 599*(4), 1039-1040. https://doi.org/10.1113/jp281197

Sunbul, M., Seckin, D., Durmus, E., Ozgen, Z., Bozbay, M., Bozbay, A., Kivrak, T., Oguz, M., Sari, I., Ergun, T., & Agirbasli, M. (2015, May). Assessment of arterial stiffness and cardiovascular

hemodynamics by oscillometric method in psoriasis patients with normal cardiac functions. *Heart Vessels, 30*(3), 347-354. https://doi.org/10.1007/s00380-014-0490-y

Syed, Z. U., & Khachemoune, A. (2011, Apr 1). Inverse psoriasis: case presentation and review. *Am J Clin Dermatol, 12*(2), 143-146. https://doi.org/10.2165/11532060-000000000-00000

Tanaka, H., Dinenno, F. A., Hunt, B. E., Jones, P. P., DeSouza, C. A., & Seals, D. R. (1998, Nov 1). Hemodynamic sequelae of age-related increases in arterial stiffness in healthy women. *Am J Cardiol, 82*(9), 1152-1155, a1110. https://doi.org/10.1016/s0002-9149(98)00578-5

Taylor, C. E., Willie, C. K., Ainslie, P. N., & Tzeng, Y. C. (2014, Jun). Assessment of human baroreflex function using carotid ultrasonography: what have we learnt? *Acta Physiol (Oxf), 211*(2), 297-313. https://doi.org/10.1111/apha.12302

Thiele, J. J., & Ekanayake-Mudiyanselage, S. (2007, Oct-Dec). Vitamin E in human skin: organ-specific physiology and considerations for its use in dermatology. *Mol Aspects Med, 28*(5-6), 646-667. https://doi.org/10.1016/j.mam.2007.06.001

Thijssen, D. H., de Groot, P., Kooijman, M., Smits, P., & Hopman, M. T. (2006, Dec). Sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *Am J Physiol Heart Circ Physiol, 291*(6), H3122-3129. https://doi.org/10.1152/ajpheart.00240.2006

A Treatise on Pathological Anatomy. (1831, Apr 1). *Med Chir Rev, 14*(28), 434-447. https://www.ncbi.nlm.nih.gov/pubmed/29919922

Tremblay, J. C., & Pyke, K. E. (2018, Mar 1). Flow-mediated dilation stimulated by sustained increases in shear stress: a useful tool for assessing endothelial function in humans? *Am J Physiol Heart Circ Physiol, 314*(3), H508-H520. https://doi.org/10.1152/ajpheart.00534.2017

Tsai, T. F., Wang, T. S., Hung, S. T., Tsai, P. I., Schenkel, B., Zhang, M., & Tang, C. H. (2011, Jul). Epidemiology and comorbidities of psoriasis patients in a national database in Taiwan. *J Dermatol Sci, 63*(1), 40-46. https://doi.org/10.1016/j.jdermsci.2011.03.002

Tsoureli-Nikita, E., Hercogova, J., Lotti, T., & Menchini, G. (2002, Mar). Evaluation of dietary intake of vitamin E in the treatment of atopic dermatitis: a study of the clinical course and evaluation of the immunoglobulin E serum levels. *Int J Dermatol, 41*(3), 146-150. https://doi.org/10.1046/j.1365-4362.2002.01423.x

Tsutsui, H., Kinugawa, S., & Matsushima, S. (2011, Dec). Oxidative stress and heart failure. *Am J Physiol Heart Circ Physiol, 301*(6), H2181-2190. https://doi.org/10.1152/ajpheart.00554.2011

Umezawa, Y., Ozawa, A., Kawasima, T., Shimizu, H., Terui, T., Tagami, H., Ikeda, S., Ogawa, H., Kawada, A., Tezuka, T., Igarashi, A., & Harada, S. (2003, Apr). Therapeutic guidelines for the treatment of generalized pustular psoriasis (GPP) based on a proposed classification of disease severity. *Arch Dermatol Res, 295 Suppl 1*, S43-54. https://doi.org/10.1007/s00403-002-0371-6

Vallbo, A. B., Hagbarth, K. E., Torebjörk, H. E., & Wallin, B. G. (1979, Oct). Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev, 59*(4), 919-957. https://doi.org/10.1152/physrev.1979.59.4.919

Vallbo, A. B., Hagbarth, K. E., & Wallin, B. G. (2004, Apr). Microneurography: how the technique developed and its role in the investigation of the sympathetic nervous system. *J Appl Physiol (1985), 96*(4), 1262-1269. https://doi.org/10.1152/japplphysiol.00470.2003

Vardi, M., Levy, N. S., & Levy, A. P. (2013, Sep). Vitamin E in the prevention of cardiovascular disease: the importance of proper patient selection. *J Lipid Res, 54*(9), 2307-2314. https://doi.org/10.1194/jlr.R026641

Victor, R. G., Leimbach, W. N., Jr., Seals, D. R., Wallin, B. G., & Mark, A. L. (1987, May). Effects of the cold pressor test on muscle sympathetic nerve activity in humans. *Hypertension, 9*(5), 429-436. https://doi.org/10.1161/01.hyp.9.5.429

Villasenor-Park, J., Wheeler, D., & Grandinetti, L. (2012, Jun). Psoriasis: evolving treatment for a complex disease. *Cleve Clin J Med, 79*(6), 413-423. https://doi.org/10.3949/ccjm.79a.11133

Vollenweider, L., Tappy, L., Owlya, R., Jequier, E., Nicod, P., & Scherrer, U. (1995, Jun). Insulin-induced sympathetic activation and vasodilation in skeletal muscle. Effects of insulin resistance in lean subjects. *Diabetes, 44*(6), 641-645. https://doi.org/10.2337/diab.44.6.641

Wadley, A. J., Veldhuijzen van Zanten, J. J., & Aldred, S. (2013, Jun). The interactions of oxidative stress and inflammation with vascular dysfunction in ageing: the vascular health triad. *Age (Dordr), 35*(3), 705-718. https://doi.org/10.1007/s11357-012-9402-1

Wang, A., & Bai, Y. (2020, Feb). Dendritic cells: The driver of psoriasis. *J Dermatol, 47*(2), 104-113. https://doi.org/10.1111/1346-8138.15184

Wang, K., Jiang, H., Li, W., Qiang, M., Dong, T., & Li, H. (2018). Role of Vitamin C in Skin Diseases. *Front Physiol, 9*, 819. https://doi.org/10.3389/fphys.2018.00819

Weinberg, J. M. (2003, Oct). An overview of infliximab, etanercept, efalizumab, and alefacept as biologic therapy for psoriasis. *Clin Ther, 25*(10), 2487-2505. https://doi.org/10.1016/s0149-2918(03)80313-2

Weiss, G., Shemer, A., & Trau, H. (2002, May). The Koebner phenomenon: review of the literature. *J Eur Acad Dermatol Venereol, 16*(3), 241-248. https://doi.org/10.1046/j.1473-2165.2002.00406.x

Weiss, S. C., Kimball, A. B., Liewehr, D. J., Blauvelt, A., Turner, M. L., & Emanuel, E. J. (2002, Oct). Quantifying the harmful effect of psoriasis on health-related quality of life. *J Am Acad Dermatol, 47*(4), 512-518. https://doi.org/10.1067/mjd.2002.122755

Weitz, G., Elam, M., Born, J., Fehm, H. L., & Dodt, C. (2001, Jan). Postmenopausal estrogen administration suppresses muscle sympathetic nerve activity. *J Clin Endocrinol Metab, 86*(1), 344-348. https://doi.org/10.1210/jcem.86.1.7138

West, X. Z., Malinin, N. L., Merkulova, A. A., Tischenko, M., Kerr, B. A., Borden, E. C., Podrez, E. A., Salomon, R. G., & Byzova, T. V. (2010, Oct 21). Oxidative stress induces angiogenesis by activating TLR2 with novel endogenous ligands. *Nature, 467*(7318), 972-976. https://doi.org/10.1038/nature09421

White, D. W., Shoemaker, J. K., & Raven, P. B. (2015, Dec). Methods and considerations for the analysis and standardization of assessing muscle sympathetic nerve activity in humans. *Auton Neurosci, 193*, 12-21. https://doi.org/10.1016/j.autneu.2015.08.004

Wollin, S. D., & Jones, P. J. (2003, Nov). Alpha-lipoic acid and cardiovascular disease. *J Nutr, 133*(11), 3327-3330. https://doi.org/10.1093/jn/133.11.3327

Wood, D. L., Sheps, S. G., Elveback, L. R., & Schirger, A. (1984, May-Jun). Cold pressor test as a predictor of hypertension. *Hypertension, 6*(3), 301-306. https://doi.org/10.1161/01.hyp.6.3.301

Yamamoto, K., Iwase, S., & Mano, T. (1992). Responses of muscle sympathetic nerve activity and cardiac output to the cold pressor test. *Jpn J Physiol, 42*(2), 239-252. https://doi.org/10.2170/jjphysiol.42.239

Ying, Z., Xie, X., Chen, M., Yi, K., & Rajagopalan, S. (2015, Jan). Alpha-lipoic acid activates eNOS through activation of PI3-kinase/Akt signaling pathway. *Vascul Pharmacol, 64*, 28-35. https://doi.org/10.1016/j.vph.2014.11.004

Yu, X. J., Li, C. Y., Dai, H. Y., Cai, D. X., Wang, K. Y., Xu, Y. H., Chen, L. M., & Zhou, C. L. (2007, Dec). Expression and localization of the activated mitogen-activated protein kinase in lesional psoriatic skin. *Exp Mol Pathol, 83*(3), 413-418. https://doi.org/10.1016/j.yexmp.2007.05.002

Yucha, C. B. (2000, Mar). Use of microneurography to evaluate sympathetic activity in hypertension: a brief review. *Appl Psychophysiol Biofeedback, 25*(1), 55-63. https://doi.org/10.1023/a:1009537506603

Zhao, Q., Gu, D., Lu, F., Mu, J., Wang, X., Ji, X., Hu, D., Ma, J., Huang, J., Li, J., Chen, J., Cao, J., Chen, C. S., Chen, J., Rice, T. K., & He, J. (2015, Nov). Blood Pressure Reactivity to the Cold Pressor Test Predicts Hypertension Among Chinese Adults: The GenSalt Study. *Am J Hypertens, 28*(11), 1347-1354. https://doi.org/10.1093/ajh/hpv035

Zhou, Q., Mrowietz, U., & Rostami-Yazdi, M. (2009, Oct 1). Oxidative stress in the pathogenesis of psoriasis. *Free Radic Biol Med, 47*(7), 891-905. https://doi.org/10.1016/j.freeradbiomed.2009.06.033

## Appendix:

***Informed Consent.***

**Appalachian State University**
**Informed Consent for Participants in**
**Research Projects Involving Human Subjects**

**TITLE:** Impact of Acute Antioxidant Supplementation on Neural Cardiovascular Control in Psoriatic Subjects

**RESEARCH TEAM:** Principal Investigator: Abigail Stickford, Ph.D. *(828-262-6799) or StickfordAS@AppState.edu; ASU Box 32071, 1179 State Farm Road, Suite 432, Boone, NC 28608.*

Co-investigators: Stephen Ratchford, Ph.D.; Nina Stute, GRA

You are being asked to be in a research study. The study is voluntary and will not affect your relationship with the study team, work, academic, or clinical staff. You may withdraw from this study *at any time* by informing the research personnel. You may decline to answer certain questions and may decide not to comply with certain procedures. However, your being in the study may depend on answering these questions or taking part in the tests. The researcher may end your role in the study without your consent if the research team determines your health or behavior adversely affects the study or increases risks beyond those outlined in this consent form. We will give you an opportunity to ask questions. All questions will be answered before you sign this consent form. Please read this form carefully. This process is called giving your *informed consent* to participate in the study.

**STUDY PURPOSE**

The **purpose** of this proposed study is to examine nerve and blood vessel function at rest and during exercise, as well as following an acute (2 week) antioxidant supplementation designed to increase exercise tolerance and decrease inflammation, oxidative stress, and muscle sympathetic nerve activity in diagnosed psoriatic subjects.

**You may be in the study if you are:**

- 18-65 years old that is currently diagnosed with Psoriasis.

**You should not be in this study if you are:**

- Younger than 18 years old
- Pregnant or trying to become pregnant
  - Currently taking medications that may influence the parasympathetic or sympathetic nervous system
  - 1) Sympathetic (adrenergic) agonists (**e.g**., phenylephrine),
  - 2) Sympatholytic drugs (adrenergic antagonists) (**e.g.,** beta-blockers such as propranolol, alpha-blockers **e.g.**, clonidine, Indoramin, Doxazosin),
  - 3) Parasympathetic agonists / Cholinergics (**e.g.**, pilocarpine), and
  - 4) Parasympathetic antagonists / Anticholinergics **(e.g.**, atropine, diphenhydramine (Benadryl), tricyclic antidepressants (Trazadone, Dresyl, Elavil, Tofranil).
    - Full exclusionary drug list with name brands will be available during informed consent process
- Prone to vasovagal episodes/fainting during needle like triggers (e.g. blood draws, shots)

**PROCEDURES**
Please read these descriptions carefully. While you may agree to participate in one or more procedures, you will always have the right to skip one or all tests in this study.

**Visit 1: Screening Day (0.5-1 hours)**
You will complete a survey about your health including: age, family health history, smoking, blood pressure and physical activity, allergies, and medications.

You will need to arrive to the lab during each testing visit having not eaten for at least 4 hours before the visit.
You will not be allowed to consume alcohol or caffeine 24 hours before each testing visit.

*Pregnancy Test (5 minutes).* Women of child-bearing age will need to take a pregnancy test. We will collect ~3 tablespoons of urine and determine your risk of being pregnant. Pregnant women must not take part in this study.

*Body Composition (10 minutes).* We will take your height and weight before you undergo a DEXA scan to determine your body composition (fat, bone, and muscle weight).

**Testing Visits 2 and 3. (3 hours/visit, 2 visits total).** You will be asked to participate in 2 experimental visits to track changes to your blood vessel, metabolic, and neural health.

All tests will be performed in Visit 2 and Visit 3. DEXA will be performed again in Visit 3.

You will need to arrive to the lab during each testing visit having not eaten for at least 4 hours before the visit.
You will not be allowed to consume alcohol or caffeine 24 hours before each testing visit.

*Pregnancy Test (5 minutes).* Women of child-bearing age will need to take a pregnancy test. We will collect ~3 tablespoons of urine and determine your risk of being pregnant. Pregnant women must not take part in this study.

*Body Composition (10 minutes).* We will take your height and weight before you undergo a DEXA scan to determine your body composition (fat, bone, and muscle weight).

*Blood Sampling (5 minutes).* We will collect a blood sample of up two tablespoons from your arm.

*Microneurography: (1-3 hours):* We will record the activity of a nerve on the side of your knee using a small electrode that looks like an acupuncture needle. LMX, Lidocaine, 4% Topical Anesthetic Cream will be used to numb participants during the microneurography procedures. You may experience small zings (twitching or tingling sensations) down your leg as we work to get this signal. Nerves are small and sometimes hard to find. Finding the right nerve will require you lay still on your back for up to 1 hour. Once we get the signal, we will see how your nerves respond to several tests while you stay relaxed in the supine position.

Subjects will be relaxed in the supine position on a tilting bed. A wrist cuff, upper arm cuff, and rubber strain-gauge will be placed on the right arm for forearm blood flow measurements.

- *Spontaneous Breathing:* You will be relaxing and breathing normally for 6 mins.
- *Controlled Breathing:* You will be relaxing
- *Valsalva Maneuver:* You will squeeze your stomach muscles to slightly increase your blood pressure by breathing into a manometer.
- *Cold Pressor Test:* You will place your hand in cold water for 2 minutes Your hand will never be in the water more than 2 minutes, however you may remove your hand at anytime and we will encourage you throughout the test.
- *Dynamic Handgrip:* You will squeeze a handgrip sensor for 2 minutes
- *Head Up Tilt:* Finally, you will be tilted on a table at 30 degrees and 60 degrees for 6 minutes each. These tests will require a series of blood pressure cuffs and equipment to monitor your heart.

*Blood Vessel Tests (30 minutes)*: We will first determine your blood pressure.

- *Flow Mediated Dilation*: We will then use a blood pressure cuff to block blood flow to your arm for up to 5 minutes and determine blood flow after we release the cuff.
- *Pulse Wave Velocity*: Then, we will assess how stiff the blood vessels are in your arm, neck, and leg by placing blood pressure cuffs on your leg and arm.

**RISKS AND DISCOMFORTS**

*Antioxidant Supplementation:* There are no foreseeable risks associated with antioxidant supplementation in the given amount administered. However, gastrointestinal discomfort has been reported in some patients. Antioxidant administration will be terminated if GI discomfort persists.

*Body Composition Test:* The risks associated with a DEXA scan include exposure to small amounts of radiation. DEXA scanning utilizes radiation to obtain an image of your body. Everyone receives a small amount of unavoidable radiation from the environment each year. Some of this radiation comes from space and some from naturally-occurring forms of radioactive water and minerals. The DEXA scan gives your body the same amount of radiation as 4 extra days' you would normally experience. If you are pregnant or trying to get pregnant, you should not participate in a DEXA scan.

*Blood sampling:* Blood draws can cause local discomfort, feeling of lightheadedness, bruising (10% chance), infection or blood clot (<0.01% chance). To minimize these risks, all blood collection procedures will be performed in a clean environment by qualified personnel. Standard precautions will be used including the cleaning of the blood draw site with alcohol,

the use of new sterile disposable needles and changing of disposable gloves in between participants.

*Blood Vessel Testing*:

- *Flow Mediated Dilation*. You may feel minor discomfort and numbness in your arm when we apply the blood pressure cuff. We will make sure the blood pressure cuff is removed as soon as we complete the test. The ultrasound probe may cause some minor discomfort from moving the probe over the skin.
- *Pulse Wave Velocity:* There are no known risks to PWV testing. However, if you become uncomfortable the test will be terminated.

*Microneurography:* There is a slight risk of temporary "pins and needles" sensation or increased sensitivity to touch in the leg following the test. Some people have said they feel some tiredness, soreness, or tingling in their leg muscles up to one week after the study. These feelings may be related to the muscle twitches which are part of the test for finding the nerve. About 7% of people may feel some aching or tingling in the area of the recording site. To reduce chances of any problems, subjects will be asked not to rub the site, stretch, or perform strenuous leg activity for 24-48 hours after the experiment. You may get light headed, have dry mouth, or feel it is hard to breath from the tests designed to increase nerve activity. You are encouraged to let us know if you feel sick, and we may end the test if we feel you may be sick from any of the tests.

During the period of time that the investigator is initially searching for the nerve using the microneurography needle, *individuals prone to vasovagal syncope may become pre-syncopal*. In training and experimental procedures in our laboratory, there has been one episode of syncope. Frequent vasovagal episodes is an exclusion criteria for this study; further, we will ask you to be verbal with any symptoms and monitor beat-by-beat blood pressure during the nerve searching.

- *Valsalva Maneuver:* For this procedure, the patient may feel light-headed or dizzy. The chances of these occurring are about 10%. This feeling will go away within 10-20 seconds once the patient stops the maneuver.
- *Cold Pressor Test*: There is minimal risk involved with this procedure; however, there is discomfort associated with this test. There are no long-term effects from performing this test. Subjects can remove their hand from the cold pressor test at any time if it is too uncomfortable.
- *Head-up Tilt*: Passive head up tilt (HUT) is regularly used to provide orthostatic challenge, a common physical stress for the human body. When a healthy person stands, blood pools in the legs, and this leads to decreases in venous return, cardiac filling pressure and output. Normal regulatory capabilities allow arterial pressure to be unaltered or slightly increased. However, the subject may feel lightheaded, tired or dizzy during head-up tilt - i.e., similar to symptoms the subject may have during prolonged standing. We will immediately stop the tilt if the subject has such sensations, and the subject will begin to feel better within seconds.

**BENEFITS**
You will not personally benefit from this study, but we hope it will provide helpful information for those suffering from Psoriasis.

**QUESTIONS**
Contact Dr. Abigail Stickford with questions, complaints, or concerns about this research. Her contact information is at the top of this form. If you have any questions about your rights as a research subject, please contact the IRB Administrator at the Appalachian State University Institutional Review Board Office at (828) 262-2692, irb@appstate.edu.

**CONFIDENTIALITY**
We will protect your confidentiality by only identifying you with a study code on all study documents, and keeping the key with your identifiable information like your name separate from the data. All records are kept in a secure, physically-locked and/or password-protected location. Any biologic samples gathered during this study will be kept coded in physically-locked locations for follow up testing related to this study. Publications resulting from the research will combine your study results with results from other people taking part in the study, without identifying information. University or government officials may need to review records from this study to ensure the study is conducted safely and legally. If this happens, they may view your personal information we have collected in this study. Identifiers might be removed from the identifiable private information and that, after such removal, the information could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from the subject or the legally authorized representative.

*Data:* The subject's information or biospecimens collected as part of the research, even if identifiers are removed,
will not be used or distributed for future research studies. Identifiers and remaining blood will be destroyed 2
years after all study analyses are complete. Deidentified data will be kept forever.

**COMPENSATION**
At this time, we cannot compensate you for your time.

**INJURY**
In the event you become injured as a result of your participation in this study, we will follow standard emergency procedures. If you get hurt or sick when you are not at the research site, you should call your doctor or call 911 in an emergency. If your illness or injury could be related to the research, tell the doctors or emergency room staff about the research study, the name of the Principal Investigator (Abigail Stickford), and provide a copy of this consent form if possible. Please call the PI (Abigail Stickford, Ph.D. 828-262-6799). You will be responsible for any costs for medical care not paid by your insurance company. No other compensation is offered by Appalachian State University. By signing this document, you are not waiving any legal rights that you have to act against Appalachian State University for injury resulting from negligence of the University or its investigators.

**SIGNATURE**
If you agree to take part in this research study and have read the information outlined above, please sign your name and indicate the date below. You will be given a copy of this signed and dated consent form for your records.

| | |
|---|---|
| **Participant, Printed Name** | **Participant, Signature** |
| **Date** | |

**I have defined and explained the study to the above volunteer.**

| | |
|---|---|
| **Consent Obtainer, Printed Name** | **Consent Obtainer, Signature** |
| **Date** | |

| | |
|---|---|
| initials | **Follow-Up Studies.** Please initial if we can hold on to your personal identifiable data and contact you for follow up studies. You can always request to have this information destroyed at a later date by contacting *Abigail Stickford.* Her contact information is at the top of this form. |

**Telephone Screening.**

Telephone Survey:
We are conducting a study to examine differences in the activity of the sympathetic nervous system (or, the system responsible for your "fight or flight" response) and the role of oxidative stress in persons who are currently diagnosed with Psoriasis. We will do a series of tests, including resting tests where we look at the activity of one of your nerves and exercise tests involving dynamic handgrip to look at the health of your blood vessels. To do this, we insert a tiny (acupuncture-like) needle in a nerve behind the knee. Once it is in place, you would be asked to stay very still (which isn't very fun!), but you shouldn't feel any pain. All data that is collected will be confidential.

**Are you interested in learning more about and potentially participating in this study?**
**Can you understand both written and oral instructions in English?**
**Are you available weekdays at any time between 7:00 am and 5:00 pm?**
**How old are you?**

If you are interested, we can have you come to the laboratory to complete some screening questionnaires to evaluate your ability to participate in the study.

**Health History Questionnaire.**

Health History Questionnaire
For Research Purposes Only

Date: _____ ID Code: _____
To be filled out by research team ONLY.

Sex: ☐ Male ☐ Female

Height: _____ Weight: _____

Year of Birth: _____ Age: _____

Ethnicity:
☐ American Indian or Alaskan Native ☐ Hispanic or Latino ☐ Other: _____
☐ Asian ☐ Native Hawaiian / Pacific Islander
☐ Black or African American ☐ White or Caucasian

Dominant Hand: ☐ Left ☐ Right

Current Occupation: _____ Previous Occupation, if retired: _____

General Questions:

Are you currently enrolled in other scientific research? ☐ Yes ☐ No

If Yes, please specify: _____

Are you willing to fast overnight? ☐ Yes ☐ No

Are you comfortable with needles or having blood drawn? ☐ Yes ☐ No

Do you have a history of fainting? ☐ Yes ☐ No

Can you tolerate lying quietly on a bed for up to 4 hours? ☐ Yes ☐ No

Can you tolerate sitting in a chair for up to 4 hours? ☐ Yes ☐ No

Do you have any orthopedic issues that would prevent you from pedaling a stationary bike or performing seated leg extensions for ~10 to 12 minutes? ☐ Yes ☐ No

Do you have a fear of confined spaces? ☐ Yes ☐ No

Physical Activity:

How many days each week are you physically activity? 1 2 3 4 5 6 7

Duration of physical activity: ☐ <15 min ☐ 15-30 min ☐ 30-45 min ☐ 45-60 min ☐ <60 min.

Intensity of physical activity: ☐ Gentle ☐ Mild ☐ Moderate ☐ Vigorous ☐ Intense

Typical physical activities:
☐ Bike ☐ Run ☐ Walk/Hike ☐ Other: _____
☐ Garden ☐ Swim ☐ Weight Lift

Exertional Symptoms: Check all of the following symptoms that apply to you when you exert yourself:

☐ Chest pain or pressure ☐ Numbness or tingling ☐ Other: _____
☐ Dizziness or light headedness ☐ Sharp pain
☐ Extreme cramping ☐ Shortness of breath

Personal Health History: Check any of the conditions you had or currently have:

| | | | |
|---|---|---|---|
| ☐ Anemia | ☐ Chronic Cough | ☐ Heart Disease | ☐ Low Blood Pressure |
| ☐ Anesthetic Problems | ☐ Contagious Disease | ☐ Heart Murmur | ☐ Low Blood Sugar |
| ☐ Anorexia / Bulimia | ☐ Diabetes | ☐ Heart Surgery | ☐ Mental Health Problems |
| ☐ Asthma / Anti-Histamines | ☐ Dialysis Treatment | ☐ Heart Valve Replacement | ☐ Mitral Valve Prolapse |
| ☐ Bleeding Tendency | ☐ Difficulty Breathing | ☐ Hepatitis | ☐ Prosthetics (Knee / Hip) |
| ☐ Blood Transfusion | ☐ Emphysema | ☐ High Blood Pressure | ☐ Thyroid Problems |
| ☐ Bronchitis | ☐ Epilepsy | ☐ Immune System Problems | ☐ TMJ Pain / Jaw Clicking |
| ☐ Cancer | ☐ Excessive Fatigue | ☐ Irregular Heart Beat | ☐ Trouble Bending Knees |
| ☐ Cardiac Pacemaker | ☐ Fainting Spells | ☐ Jaundice | ☐ Tuberculosis |
| ☐ Cardiovascular Disease | ☐ Hay Fever / Sinus Problems | ☐ Kidney Trouble | ☐ Tumor / Growth |
| ☐ Chemotherapy Treatment | ☐ Heart Attack | ☐ Liver Disease | |

Other: _____

If Yes to any of the above, please specify: _____

_____

_____

Current Symptoms: Check any of the following symptoms that you currently have:

| | | | |
|---|---|---|---|
| ☐ Ankle Swelling | ☐ Coughing, Wheezing | ☐ Joint Pain | ☐ Tingling Feet |
| ☐ Back Pain | ☐ Difficulty Breathing | ☐ Non-Healing Skin Sores | ☐ Tingling Hands |
| ☐ Blood in Stool | ☐ Dizziness | ☐ Numb Feet | ☐ Trouble Sleeping |
| ☐ Burning Feet | ☐ Excessive Thirst | ☐ Numb Hands | ☐ Vision Problems |
| ☐ Burning Hands | ☐ Frequent Urination | ☐ Paralysis | |
| ☐ Chest Congestion | ☐ Heart Palpitations | ☐ Sudden Weight Gain | |
| ☐ Chest Pain / Pressure | ☐ Infrequent Bowel Habits | ☐ Sudden Weight Loss | |

Other: _____

If Yes to any of the above, please specify: _____

_____

_____

Allergies: _____

_____

Do you use any allergy medication(s)? ☐ Yes ☐ No

If Yes, please specify: _____

Have you ever had a stress test? ☐ Yes ☐ No

If Yes, please specify: _____

Have you had any major surgery in the past 6 months? ☐ Yes ☐ No

If Yes, please specify: _____

Medications: Please list *all medications* you are taking or have recently taken, *dosage*, and *reason*:

_____

_____

_____

_____

Do you use any over-the-counter drugs or supplements? ☐ Yes ☐ No

If Yes, please specify: _____

Are you *able* to forego any the above listed drugs for the duration of this study? ☐ Yes ☐ No

Are you *willing* to forego any the above listed drugs for the duration of this study? ☐ Yes ☐ No

For Women:

Are you currently pregnant? ☐ Yes ☐ No

Are you currently trying to become pregnant? ☐ Yes ☐ No

Are you currently using contraceptives? ☐ Yes ☐ No

If Yes, please specify: _____

What is your menopausal status? ☐ Pre ☐ Peri ☐ Post

If Post-menopausal, indicate for how long: _____

Onset of menopause was: ☐ Natural ☐ Surgical ☐ Other

Have you or are you on hormone replacement therapy? ☐ Yes ☐ No

If Yes, please specify: _____

Family Health History: Check all of the following which apply to your immediate (parents / siblings) family:

| | | | |
|---|---|---|---|
| ☐ Arthritis | ☐ Cancer | ☐ Heart Disease | ☐ High Blood Press |
| ☐ Asthma | ☐ Diabetes | ☐ Heart Surgery | ☐ Obesity |
| ☐ Balloon angioplasty/stent | ☐ Heart Attack | ☐ High Blood Cholesterol | ☐ Osteoporosis |

**The information that is obtained during my participation will be treated as privileged and confidential. It is not to be released or reveled to any person without my written consent. The information obtained, however, may be used for statistical analysis or scientific purpose with my right to privacy retained.**

**I understand that accurate information about my health history is required to determine the safety of the study participation being asked of me. I declare that information provided on this health history questionnaire is true and accurate to the best of my information, knowledge, and belief.**

**Signature:**_____ **Date:** _____

## IRB Approval:

**From:** Dr. Andrew Shanely, IRB Chairperson
**RE:** Notice of IRB Approval by Full Board Review
**Grant Title**: **Sponsors:** National Psoriasis Foundation

**STUDY #:** 20-0185
**STUDY TITLE:** Impact of Acute Antioxidant Supplementation on Neural Cardiovascular Control in Psoriatic Subjects
**Submission Type:** Initial
**Approval Date:** 4/21/2020
**Expiration Date of Approval:** 4/20/2021

**NOTE:** This project, like all exempt and non-exempt research with human subjects at Appalachian State University, is subject to other requirements, laws, regulations, policies, and guidelines of the University and the state of North Carolina. As of April 2, 2020 and until further notice, this includes the requirement by the Office of Research to pause all research in response to the COVID-19 pandemic and the North Carolina statewide stay-at-home order. Please see the full requirement on the Research Protections website, as well as answer to any questions you may have.
The Institutional Review Board (IRB) reviewed this study at a convened meeting and approved this study for the period indicated above. IRB approval is limited to the activities described in the IRB approved materials, and extends to the performance of the described activities in the sites identified in the IRB application. In accordance with this approval, IRB findings and approval conditions for the conduct of this research are listed below.

***National Psoriasis Foundation Grant Submission;***
***Cover Letter***

COLLEGE OF HEALTH SCIENCES
Exercise Science
APPALACHIAN STATE UNIVERSITY

January 13, 2020

ASU Box 32071
Department of Health & Exercise Science
Leon Levine Hall of Health Sciences
Boone, NC 28608-2071

To the National Psoriasis Foundation,

Please find enclosed all applicable required materials for my National Psoriasis Foundation 2020 Early Career Research Grant submission, entitled "Impact of Acute Antioxidant Supplementation on Neural Cardiovascular Control in Psoriatic Subjects." As a first-year graduate student with plans to obtain my doctorate and work towards becoming a **leader in the scientific research community**, I am an excellent fit for the goals of this award.

Our proposed project will be **the first to directly measure neural sympathetic activation**, in addition to inflammatory markers and vascular function, in psoriatic patients following antioxidant supplementation. We hypothesize that this acute supplementation will reduce inflammation and subsequently lower sympathetic activation and improve vascular function. These hypothesized changes would have long-term beneficial implications for the patient's functional abilities and quality of life. Our collaborative laboratories (i.e., my mentors Dr. Abigail Stickford and Dr. Stephen Ratchford) at Appalachian State University are unique in their **capability to carry out all aspects of this proposed project**.

There are no known reviewers with conflicts of interest regarding this research proposal.

I appreciate your consideration and look forward to hearing from you at your earliest convenience.

Sincerely,

Nina Stute

***National Psoriasis Foundation Grant Submission;***

***Specific Aims Page*** *(clipped to show only specific aims)*

Running Head: AOx AND NEURAL CARDIOVASCULAR CONTROL IN PSORIASIS

**Specific Aims:**

The **purpose** of this proposed study is to examine muscle sympathetic nerve activity (MSNA) and vascular function at rest and during exercise, as well as following an acute antioxidant supplementation designed to dampen vascular inflammation, oxidative stress, and MSNA in diagnosed psoriatic subjects.

Specific aims and hypotheses:

**Specific Aim 1:** Identify the role of oxidative stress on muscle sympathetic nerve activity in patients with psoriasis. Investigate the ability of acute antioxidant administration (Vitamin C, E, and alpha lipoic acid) to reduce oxidative stress and subsequent MSNA in patients with psoriasis.

- Primary Hypothesis: Vasomotor tone and Oxidative-stress/inflammatory biomarkers (TNF-α, CRP, IL-1, IL-6, VEG-F, and MDA) will be increased in psoriatic subjects but will decrease post acute antioxidant supplementation.

**Specific Aim 2:** Compare vascular function before and after acute antioxidant supplementation in psoriatic subjects.

- Primary Hypothesis: Decreased arterial compliance and endothelium-dependent flow mediated dilation will be observed in psoriatic subjects to a higher degree than undiagnosed persons.

***National Psoriasis Foundation Grant Submission;***

***Notifications***

*Email notification:*

**From:** Stacie Bell <sbell@psoriasis.org>
**Subject: NPF 2020 Grant Notification**
**Date:** June 30, 2020 at 5:10:09 PM EDT
**To:** "stutenl@appstate.edu" <stutenl@appstate.edu>
**Cc:** Jackie Domire <jdomire@psoriasis.org>, Stacie Bell <sbell@psoriasis.org>

Dear Dr. Stute,

Thank you for your submission to the National Psoriasis Foundation Early Career Research Grant. I am sorry to inform you that your application was not selected for funding. The Foundation receives many strong applications and regrets that we are unable to fund all of the meritorious projects.

Attached to this email, you can find a summary statement which includes written comments provided during peer review of your application.

We would be happy to provide additional feedback on the outcome of your application, answer any questions, or address any concerns regarding this funding decision. On behalf of the National Psoriasis Foundation, thank you for your interest in and commitment to psoriatic disease research.

Sincerely,

Stacie

Stacie Bell, PhD
**Chief Scientific and Medical Officer**

National Psoriasis Foundation
6600 SW 92nd Avenue, Suite 300
Portland, OR 97223
503-546-5558 (office) | 303-929-3024 (Cell)
sbell@psoriasis.org | psoriasis.org/give

*Attachment of scores (cover page, only, shown):*

NATIONAL PSORIASIS FOUNDATION®

# 2020 Research Grants

## Review Summary

**Application Type:**
Early Career Research Grants

**Principal Investigator:**
Nina Stute

**Project Title:**
Impact of Acute Antioxidant Supplementation on Neural Cardiovascular Control in Psoriatic Subjects

**Outcome:**
Not-Funded

**Explanation:**
In an effort to fund grants that are both scientifically meritorious and aligned with NPF's priorities, NPF conducts a three-stage peer-review process:

| Step | Review Activity | Output |
|---|---|---|
| 1 | Preliminary scientific review by three peer-reviewers | Numerical scores and brief rationale |
| 2 | Discussion and final assessment by scientific peer-review committee | Numerical scores, discussion notes, and detailed rationale |
| 3 | Discussion and assessment by NPF Research Committee | Discussion notes and funding determination |

All grants undergo the first step of the review process, but only a fraction move on to the second step, and from there only a fraction move on to the third step. The extent of the comments contained in the following pages of this document depends on the step to which this application advanced. All numerical scores follow the 9-point NIH scoring system:

| | | | |
|---|---|---|---|
| **High Impact:** | 1 – Exceptional | 2 – Outstanding | 3 – Excellent |
| **Medium Impact:** | 4 – Very Good | 5 – Good | 6 – Satisfactory |
| **Low Impact:** | 7 – Fair | 8 – Marginal | 9 - Poor |

**The Journal of Physiology Journal Club Article Submission;**

The Journal of Physiology

*J Physiol* 599.4 (2021) pp 1039–1040



JOURNAL CLUB

## Pushing the needle forward on the relationship between autoimmunity and autonomic dysfunction

**Nina L. Stute and P. J. Koopmans**

*Department of Health & Exercise Science, Appalachian State University, Boone, NC, USA*

Email: stutenl@appstate.edu

Edited by: Harold Schultz & Vaughan Macefield

Linked articles: This Journal Club article highlights an article by Peçanha *et al.* To read this article, visit https://doi.org/10.1113/JP280892.

Immune-mediated diseases (IMDs) impact 4% of the global population, making them of great clinical and economic importance. They are a physical, emotional and economic burden for patients, oftentimes decreasing quality of life in multiple facets. The IMD burden impacts more than just patients: it overflows into the medical community, as the great majority of IMDs are accompanied by numerous comorbidities.

Rheumatoid arthritis (RA) falls under the IMD umbrella as a chronic-inflammatory autoimmune disease: it is characterized by systemic inflammation, severe joint pain and an increased risk of serious comorbidities. Of these comorbid conditions, the most alarming is cardiovascular disease (CVD), which accounts for 40–50% of deaths in RA. Patients with RA experience high incidence of myocardial infarction and cerebrovascular stroke. The proposed mechanisms connecting RA and CVD are wide ranging: chronic systemic inflammation, which can wreak havoc on endothelial function; adverse interactions and side effects of disease-altering drugs and biologic therapies; as well as lifestyle and environmental factors that predispose patients to a plethora of CVD risk factors.

Over the past five decades, using the technique of microneurography to investigate afferent and efferent nerve impulses has been a staple in autonomic function research. As such, this gold standard assessment of autonomic function, which allows for direct recording of muscle sympathetic nerve activity (MSNA), has been employed to elucidate the ambiguous relationship between RA and CVD. RA is hypothesized to be somewhat unique, as autonomic dysfunction may occur *prior* to RA development, as found by observational studies (Koopman *et al.* 2016). Autonomic dysfunction, like that seen in RA, wherein MSNA is elevated in both basal and reactivity measures, is recognized as one of the mechanisms contributing to adverse cardiovascular outcomes in RA. In a disease where early detection can save one from irreparable joint damage, such information is powerful in helping identify risk factors earlier rather than later.

Autonomic and haemodynamic reactivity to exercise is complex. The muscle metaboreflex, which responds to an accumulation of metabolites in exercising skeletal muscle when $O_2$ demands are not met, sparks a reflex to increase blood pressure (BP). An increase in MSNA during muscle metaboreflex activation, which can be stimulated via post-exercise ischaemia (PEI), has been shown to contribute to compromised haemodynamic control in various disease states (heart failure, hypertension, etc.). Isolating the metabolic portion of the exercise pressor response provides valuable insight into the neural and metabolic mechanisms contributing to RA. While autonomic dysfunction is clearly a key player in RA, just how much it pervades homeostatic processes has been unclear.

This gap in the literature was addressed in a recent article in the *Journal of Physiology* by Peçanha *et al.* (2021). The authors recruited ($n = 33$) post-menopausal females with RA diagnoses and ($n = 10$) age- and CVD risk-matched controls (CON) for their cross-sectional study. The main findings of this work were that the RA group showed both augmented sympathetic and pressor responses during exercise, which were sustained during PEI. To little surprise, the RA group also had elevated MSNA and compromised baroreflex sensitivity during basal measures.

Importantly, CVD risk factors did not differ between the groups. The RA group had a mean ± SD disease duration of 20 ± 12 years with 97% of RA patients taking disease-modifying anti-rheumatic drugs and 48% using biologic agents. Participants underwent three laboratory visits in total: (1) a clinical evaluation consisting of medical history, pain levels via a visual analogue scale (VAS), and disease activity, as well as a 12 h fasted blood sampling (30 ml) for a wide range of biomarkers; (2) a $\dot{V}_{O_2}$ maximal graded exercise test, conducted on a treadmill with incremental increases in speed and grade each minute until voluntary cessation; and (3) a battery of autonomic function tests that consisted of maximal voluntary contraction (MVC) isometric knee extension of the left leg, 15 min of basal measurements, 3 min of isometric knee extension at 30% MVC, directly followed by 2 min of cuff occlusion (PEI), all while remaining supine and recording MSNA from the non-exercising leg in the peroneal nerve at the fibular head.

Peçanha *et al.* found that the RA group presented greater VAS pain ($P = 0.02$) and inflammatory biomarkers (C-reactive protein, $P = 0.01$; interferon-$\gamma$, $P = 0.03$; interleukin (IL)-10, $P = 0.03$; IL-1ra, $P = 0.02$; IL-8, $P = 0.01$; and monocyte chemotactic protein 1, $P = 0.01$) when compared to the CON group. There were no significant differences between groups for resting haemodynamic measures and heart rate variability. However, the RA group had significantly greater basal MSNA burst frequency than the CON group (bursts/min, RA: 33 ± 14 *vs.* CON: 20 ± 13, $P = 0.03$) and burst incidence (bursts/100 heart beats, RA: 50 ± 24 *vs.* CON: 31 ± 20, $P = 0.04$). Similarly, during exercise there were no differences in heart rate or root mean square of the successive R-R intervals between groups. However, mean arterial pressure ($P = 0.003$) and MSNA ($P = 0.03$) during both exercise and PEI were significantly higher in the RA group when compared to CON. Indeed, Peçanha *et al.* also found that the baroreflex was impaired in the RA group: lower baroreflex effectiveness index ($P = 0.002$) and trend to lower cardiac baroreflex sensitivity in RA compared to CON ($P = 0.06$).

The authors should be commended for their thorough investigation into this clinical population. They brought forward many powerful points, the most impactful of which, in our novice opinion, is that persons with RA may be experiencing multiple BP dysregulations, or surges, throughout the day, further amplifying the risk of sustaining a severe cardiovascular



DOI: 10.1113/JP281197

event. Activities as simple as getting the mail and taking a shower could be a stressor that spikes MSNA, in turn causing harmful and uncontrolled BP fluctuations. Furthermore, this work strengthens our understanding of the relationship between autoimmune disease and autonomic dysfunction. RA is similar to various IMDs, such as Crohn's disease, ulcerative colitis, endometriosis, psoriasis and more, in cytokine storms, inflammatory pathways and adverse cardiovascular outcomes, and this begs the question: how deep rooted is autonomic dysfunction in disease pathogenesis under the IMD umbrella?

Along with the limitations listed in the discussion, the authors may have benefitted from utilizing the VAS pain scale during the exercise protocol, given the limits of the clinical population. Chronic pain – chiefly during exercise – certainly may compound with other factors to decrease exercise tolerance. The addition of the VAS pain scale during and after the exercise protocol could have proved valuable in comparing exercise tolerance between RA and CON groups, as well as aiding in the further solidification of the possible role, or lack thereof, of pain in exercise tolerance between groups.

One of the most universal strategies for effectively reducing risk of comorbidities common to RA is physical activity. Exercise, when combined with proper drug therapy, is essential in aiding the control of systemic inflammation, and decreasing endocrine-active central adiposity, oxidative stress and CVD risk. However, sufferers of RA have a steep mountain to climb in regards to exercise. Those with sustained and moderate to high disease activity often experience arthrogenic inhibition to the muscles surrounding affected joints, as well as peripheral deafferentation. These changes in sensory feedback could modify proprioception and have an impact on subsequent motor patterns, further decreasing exercise capacity and general physical activity tolerance.

Furthermore, there is sufficient evidence that shows correctly designed dynamic strength programmes, consisting of both resistance and aerobic training, are effective in improving physical function and quality of life in RA patients. While dynamic strength training is crucial for post-menopausal women, as loss of bone mineral density and sarcopenia are cause for concern, it is especially relevant in a high disease state like that of RA, where appropriately programmed exercise regimens can decrease CVD risk as well as RA severity and activity (Hakkinen *et al*. 2001; Stavropoulos-Kalinoglou *et al*. 2013).

Along with the aforementioned suggestion of expanding these findings by investigating the metaboreflex in similar disease states, another avenue that may prove important would be observation of the neurovascular transduction of sympathetic activity into vascular tone. It has been established that as females age, neurovascular transduction of MSNA is increased: high sympathetic activity leads to a higher level of vasoconstriction (Briant *et al*. 2016). However, for males, this is not the case. Of great interest would be comparing pre- *vs*. post-menopausal females as well as males *vs*. females to help elucidate the cardioprotective benefits of oestrogen and its impact on disease pathogenesis. This may be part of the reason for increased prevalence of RA in older females than males.

In conclusion, getting RA patients to be physically active early could keep them out of cardiac rehabilitation later. The progressive joint damage and loss of function develops early at disease onset in RA. As such, early diagnosis and appropriate drug therapy are essential for favourable patient outcomes; however, physical activity should be considered an integral aspect to patient care for both prevention and recovery. As previously stated, this work further supports the existence of a relationship between autoimmune disease and autonomic dysfunction. Additional studies are warranted to investigate autonomic (dys)function's role in similar disease states across the sexes: a possible common denominator in an expansive and expensive sea of information.

## References


Briant LJ, Burchell AE, Ratcliffe LE, Charkoudian N, Nightingale AK, Paton JF, Joyner MJ & Hart EC (2016). Quantifying sympathetic neuro-haemodynamic transduction at rest in humans: insights into sex, ageing and blood pressure control. *J Physiol* **594**, 4753–4768.

Hakkinen A, Sokka T, Kotaniemi A & Hannonen P (2001). A randomized two-year study of the effects of dynamic strength training on muscle strength, disease activity, functional capacity, and bone mineral density in early rheumatoid arthritis. *Arthritis Rheum* **44**, 515–522.

Koopman FA, Tang MW, Vermeij J, de Hair MJ, Choi IY, Vervoordeldonk MJ, Karemaker JM & Tak PP (2016). Autonomic dysfunction precedes development of rheumatoid arthritis: a prospective cohort study. *EBioMedicine*, **6**, 231–237.

Peçanha T, Meireles K, Pinto AJ, Rezende DAN, Iraha AY, Mazzolani BC, Smaira FI, Sales ARK, Bonfiglioli K, de Sá-Pinto AL, Lima FR, Irigoyen MC, Gualano B & Roschel H (2021). Increased sympathetic and haemodynamic responses to exercise and muscle metaboreflex activation in post-menopausal women with rheumatoid arthritis. *J Physiol*, **599**, 927–941.

Stavropoulos-Kalinoglou A, Metsios GS, Veldhuijzen van Zanten JJ, Nightingale P, Kitas GD & Koutedakis Y (2013). Individualised aerobic and resistance exercise training improves cardiorespiratory fitness and reduces cardiovascular risk in patients with rheumatoid arthritis. *Ann Rheum Dis* **72**, 1819–1825.


## Additional information

### Competing interests

No competing interests declared.

### Author contributions

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.


### Funding

None.

### Acknowledgements

We apologize for not citing all relevant works due to reference limitations. We would like to thank Dr. Abigail S. L. Stickford for her encouragement and review of the manuscript.

### Keywords

autoimmune disease, baroreflex, microneurography, muscle sympathetic nerve activity, post-exercise ischaemia, rheumatoid arthritis

## Vita

Nina Lawrene Stute was born in Dayton, Ohio, USA to Susan Stewart-Stute and Michael Stute. She graduated from The Ohio State University in Columbus Ohio in July 2019 with a B.S.. Directly after, she entered Appalachian State University in Boone North Carolina to study Exercise Science and accepted a research assitantship with Dr. Abigail Stickford. The M.S. was awarded in May 2021.

Nina still has no idea where she is going to school next.